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Effects and Fate of Sewage Chlorination Products in Phytoplankton

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The effects of seven stable chloro-organic compounds formed during chlorination of domestic waste-water on the growth of selected fresh-water and marine phytoplankton were determined. The uptake and metabolism of selected chloro-organic chemicals by the phytoplankton were also investigated.

3-Chlorophenol, 3-chlorobenzoic acid, 4-chlororesorcinol, 5-chlorouracil, 5-chlorouridine, 6-chloroguanine or 8-chlorocaffeine at a concentration of 0.1 ppm, alone or in combinations of up to 4 chemicals, had no significant effect on the yield of Scenedesmus obliquus, Selenastrum capricornutum, Microcystis aeruginosa, Dunaliella tertiolecta, Skeletonema costatum, Thalassiosira pseudonana, and Porphyridium sp. 4-Chlororesorcinol and 5-chlorouracil were taken up by certain species but neither chemical was accumulated to a high level. The uptake of chlororesorcinol was considerably greater than that of chlorouracil. The uptake of 3-chlorobenzoic acid by the phytoplankton was negligible.

4-Chlororesorcinol was readily degraded in aqueous solution by the action of simulated sunlight and both Skeletonema and Selenastrum took up chlororesorcinol as well as its photodegradation products from the medium.

Neither Skeletonema nor Selenastrum were able to metabolize 4-chlororesorcinol in the dark but appeared to transform it to some extent into more polar material(s) in the light.

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FOREWORD

Effective regulatory and enforcement actions by the Environmental Protection Agency would be virtually impossible without sound scientific data on pollutants and their impact on environmental stability and human health. Responsibility for building this data base has been assigned to EPA's Office of Research and Development and its 15 major field installations, one of which is the Corvallis Environmental Research Laboratory (CERL).

The primary mission of the Corvallis Laboratory is research on the effects of environmental pollutants on terrestrial, freshwater, and marine ecosystems; the behavior, effects and control of pollutants in lake systems; and the development of predictive models on the movement of pollutants in the biosphere.

This report examines the effects of seven major chloro-organic compounds, typically formed during chlorination of sewage effluent, on the growth of seven selected species of marine and freshwater phytoplankton. Despite the lack of a discernible effect on their growth at a maximum concentration of 0.1 ppm, there was a substantial variance between some of the algae in the uptake and metabolism of some of the compounds.

This information can be important in assessing the potential for artificially induced floral changes through species selection or altered grazing pressure through the transfer of these accumulated compounds through the food chain.

These results more clearly define areas of program interest for further research that can be useful for determining tolerable levels of these compounds in wastewater discharges.

J. C. McCarty
Acting Director, CERL

ABSTRACT

The effects of seven stable chloro-organic compounds formed during chlorination of domestic waste-water on the growth of selected fresh-water and marine phytoplankton were determined. The uptake and metabolism of selected chloro-organic chemicals by the phytoplankton were also investigated.

3-Chlorophenol, 3-chlorobenzoic acid, 4-chlororesorcinol, 5-chlorouracil, 5-chlorouridine, 6-chloroguanine or 8-chlorocaffeine at a concentration of 0.1 ppm, alone or in combinations of up to 4 chemicals, had no significant effect on the yield of Scenedesmus obliquus, Selenastrum capricornutum, Microcystis aeruginosa, Dunaliella tertiolecta, Skeletonema costatum, Thalassiosira pseudonana, and Porphyridium sp. 4-Chlororesorcinol and 5-chlorouracil were taken up by certain species but neither chemical was accumulated to a high level. The uptake of chlororesorcinol was considerably greater than that of chlorouracil. The uptake of 3-chlorobenzoic acid by the phytoplankton was negligible.

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Neither Skeletonema nor Selenastrum were able to metabolize 4-chloro-resorcinol in the dark but appeared to transform it to some extent into more polar material(s) in the light.

This report was submitted in fulfillment of EPA Grant R 804-938-010 by Syracuse Research Corporation under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period November 1, 1976 to February 28, 1978, and work was completed as of February 28, 1978.

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LIST OF ABBREVIATIONS

CB --	3-chlorobenzoic acid
CC --	8-chlorocaffeine
CG --	6-chloroguanine
CP --	3-chlorophenol
CR --	4-chlororesorcinol
CU --	5-chlorouracil
CUD --	5-chlorouridine

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SECTION 1

INTRODUCTION

Chlorination is a widely-used practice for disinfecting municipal waste-waters or combined municipal-industrial wastes before they are discharged into receiving waters. The quantity of chlorine used for sewage treatment is expected to increase as municipalities initiate or upgrade their treatment to include chlorination as a disinfectant to meet state or local standards in lieu of not yet widely accepted alternatives such as ozonation or bromination. Additionally, an increasing number of industries have been required to provide waste-water treatment which often includes effluent chlorination.

It is conceivable that some organic compounds may escape secondary treatment or be only partially degraded and as a result they may react with chlorine, yielding persistent, potentially toxic organic compounds. Recently, Glaze et al. (13) and Jolley (17,18) have shown that chlorine-containing organic compounds are produced when sewage effluents are chlorinated. Jolley (18) has identified 17 stable, chlorine-containing organic compounds in domestic waste-water effluent which had been chlorinated to a 1 to 2 mg/ℓ combined chlorine residual. Some of these compounds included chlorinated phenols, aromatic acids, purines and pyrimidines. Barnhart and Campbell (2), in studies of industrial waste-water effluents observed that chlorine reacted readily with phenol, m-cresol, and aniline under conventional effluent treatment conditions. Based on the assumption that a total of 100,000 tons of chlorine is used annually in the U.S. for disinfecting waste-water, Jolley (18) estimated that approximately 5,000 tons of stable chlorine-containing organic compounds would be released to the receiving water ecosystem annually.

The presence of chloro groups is known to render benzenoid compounds more resistant to microbiological degradation (1), which suggests a potential for accumulation of these compounds in receiving waters. The introduction of chlorinated organics into the aquatic environment is of great environmental concern because of their potential toxicity to various organisms. In order to evaluate fully the impact of waste-water chlorination on the aquatic environment, it is necessary that we know the effects and fate of the chlorination products in the biota. The toxicity of residual chlorine on aquatic life has been extensively investigated and was recently reviewed by Brungs (5). However, very little information is available on the effects of stable chlorine-containing organic compounds that may have been produced during the chlorination process. Gehrs et al. (12) have recently reported that both 5-chlorouracil and 4-chlororesorcinol, which are among the constituents in chlorinated effluents, decreased the hatchability of carp eggs at concentrations as low as 1 ppb.

Phytoplankton constitute a vital part of the aquatic biota. An adverse effect on the growth of phytoplankton, which represent the first link in the aquatic food chain, may have adverse effects on the entire ecosystem. Algae may also play an important role in determining the fate of chloro-organics in the aquatic ecosystem. They may remove the chemicals from the environment by adsorption and/or absorption and may subsequently metabolize them. An accumulation of chemicals or their transformation products by phytoplankton is of ecological significance because these chemicals may be transferred to the higher trophic levels with potentially deleterious effects.

In our recent studies on the effects of 3-chlorophenol, 4-chloro-resorcinol, 5-chlorouracil, and 3-chlorobenzoic acid on selected species of marine phytoplankton (27), we observed that the chemicals up to a concentration of 1 ppm had either a slight effect or no effect on the growth of the organisms. However, no information on the toxicity of these chemicals to fresh-water phytoplankton is available. This study was undertaken to investigate further the effects and fate of selected stable organic compounds produced during chlorination of sewage effluents on both marine and fresh-water phytoplankton. The results will provide information needed for establishing guidelines for the discharge of chlorine-treated waste-waters into the aquatic ecosystems.

Specific Objectives:

1. To study the effect of selected sewage-effluent chlorination products on the growth of fresh water and marine phytoplankton.

2. To study the interaction among the test chemicals by examining the effect of one chemical in the presence of the other chemicals.

3. To study the effect of nutrient levels on the toxicity of the test chemicals to the phytoplankton.

4. To study the uptake of the test chemicals by fresh-water and marine phytoplankton.

5. To study the metabolism of the test chemicals by the phytoplankton.

SECTION 2

CONCLUSIONS

A number of chloro-organic compounds produced during chlorination of waste-water, such as 3-chlorophenol, 3-chlorobenzoic acid, 4-chlororesorcinol, 5-chlorouracil, 5-chlorouridine, 6-chloroguanine and 8-chloroguanine at a concentration of 0.1 ppm alone or in combinations of up to four chemicals, had no significant effect on the yield of several fresh-water and marine phytoplankton species.

4-Chlororesorcinol and 5-chlorouracil were taken up by certain species but neither chemical was accumulated to a high level. The uptake of 3-chlorobenzoic acid by the phytoplankton was negligible.

Neither Skeletonema costatum nor Selenastrum capricornutum were able to metabolize 4-chlororesorcinol in the dark but appeared to transform it to some extent into more polar material(s) in the light.

These findings suggest that simple mono-chlorinated organic chemicals, at the concentrations tested, are not expected to have any significant effect on growth of phytoplankton in the aquatic environment.

SECTION 3

RECOMMENDATIONS

Our studies have shown that a number of simple mono-chlorinated organic compounds formed during chlorination of sewage effluents have no significant effect on the yield of fresh-water and marine phytoplankton. However, in order to fully assess the environmental impact of these chemicals we recommend that:

1. Studies should be undertaken to determine the toxicity of the chemicals to aquatic invertebrates and fish.

2. Uptake and metabolism of the chemicals by fish and Daphnia should be investigated.

3. The role of various processes (biodegradation, photodegradation, adsorption to sediment) which may determine the environmental behavior of these chemicals should be studied. The rates and products of environmental degradation of these chemicals should be determined to assess their fate in the ecosystem.

SECTION 4

MATERIALS AND METHODS

CHEMICALS

The chemicals tested for toxicity to phytoplankton included 3-chlorobenzoic acid, 8-chlorocaffeine, 6-chloroguanine, 3-chlorophenol, 4-chlororesorcinol, 5-chlorouracil, and 5-chlorouridine (Figure 1). These chemicals were among the 17 stable chlorine-containing organic compounds which Jolley (18) identified in chlorinated sewage effluents. These chemicals were specifically selected because their concentrations in chlorinated sewage-effluents were relatively high compared to those of other chlorine-containing organic compounds.

3-Chlorobenzoic (99%), 3-chlorophenol (97% or greater), and 4-chlororesorcinol (99%) were purchased from Aldrich Chemical Company; 5-chlorouracil and 5-chlorouridine (both Grade A) from Calbiochem; 6-chloroguanine from Sigma Co., and 8-chlorocaffeine (98-99%) from ICN. Uniformly ring-labeled ^{14}C-3-chlorobenzoic acid (sp. activity 4.6mCi/mM) and ^{14}C-4-chlororesorcinol (sp. activity 9.5mCi/mM) were purchased from the California Bionuclear Corporation, Sun Valley, California. ^{14}C-5-Chlorouracil was synthesized in our laboratory by chlorinating ^{14}C-uracil using the procedure described by West and Barrett (30). The ^{14}C-chlorouracil produced was then purified by high-pressure liquid chromatography (HPLC) using a Waters Associates (Milford, Mass.) liquid chromatograph (model M6000A) equipped with a U.V. detector (Schoeffel Instrument Corp., Westwood, N.J., model GM770). A 4 mm (i.d.) X 30 cm column packed with μ Bondapak C18 reversed phase medium (Waters Associates) was utilized. Chlorouracil was detected by its absorption at 282 μm. The solvent system used was methanol:water (1:7 v/v) which provided excellent separation of the unreacted uracil from 5-chlorouracil. The reaction solution was injected directly into the HPLC; the fraction containing 5-chlorouracil, as indicated by the UV detector, was collected and stored in freezer for subsequent use. Analysis of this fraction by HPLC showed that ^{14}C-chlorouracil had a purity of greater than 99%.

ALGAL SPECIES

The fresh-water phytoplankton species tested were *Microcystis aeruginosa* (Cyanophyta), *Scenedesmus obliquus* (Chlorophyta), and *Selenastrum capricornutum* (Chlorophyta). The marine phytoplankton were *Dunaliella tertiolecta* (DUN clone) (Chlorophyta), *Skeletonema costatum* (SKEL clone)(Bacillariophyta), *Thalassiosira pseudonana* (CN clone)(Bacillariophyta) and *Porphyridium* sp. (Rhodophyta). The stock cultures of the fresh-water algae were obtained

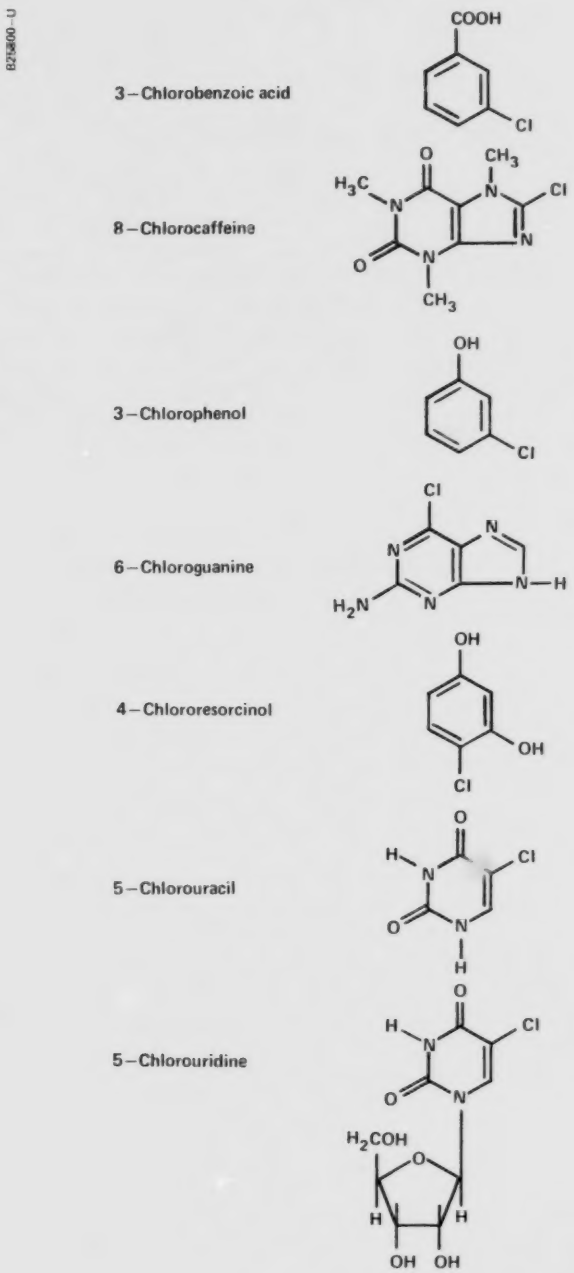


Figure 1. Structures of chloro-organic compounds involved in this study.

from the Culture Collection of Algae, University of Texas at Austin. The cultures of marine species were obtained from the Woods Hole Oceanographic Institute, Woods Hole, Mass. All cultures were axenic except for Microcystis aeruginosa.

CULTURING OF ALGAE

Scenedesmus obliquus and Selenastrum capricornutum were grown axenically in the medium specified in the Algal Assay Procedure, EPA, 1971 (28). Non-axenic Microcystis aeruginosa was grown in a modified Gorham's medium consisting of the following ingredients (μmoles/ℓ) in glass distilled water: $NaNO_3$ (2000), $MgCl_2$ (200), $MgSO_4 \cdot 7H_2O$ (200), $CoCl_2$ (200), K_2HPO_4 (100), $FeCl_3$ (4), NaEDTA (20), H_3BO_3 (40), $MnCl_2 \cdot H_2O$ (2), $ZnCl_2$ (0.0008), $CoCl_2$ (0.08). The initial pH of the medium was 7.5. All four fresh-water species were grown on a reciprocating shaker (100 oscillations/min) under controlled environmental conditions; 21°C ± 1°C, and 400 ft-c continuous illumination.

Skeletonema costatum, Thalassiosira pseudonana, and Dunaliella tertiolecta were grown axenically in Modified Burkholder's artificial seawater medium recommended by the Environmental Research Laboratory, USEPA, Corvallis, Oregon (1974). Porphyridium sp. was grown axenically in Modified Burkholder's medium supplemented with the following: 16.48 mg N/1 as $NaNO_3$, 4.45 mg P/1 as K_2HPO_4, 4.94 mg Si/1 as $Na_2SiO_3 \cdot 9H_2O$, 1 μg/1 biotin, 0.2 mg/1 thiamine-HCl and 1 ml/1 NAAM (29) trace metal mix (0.1856 g H_3BO_3; 0.416 g $MnCl_2 \cdot 4H_2O$; 0.032 g $ZnCl_2$; 1.428 mg $CoCl_2 \cdot 6H_2O$; 0.0214 mg $CuCl_2 \cdot 2H_2O$; 7.26 mg $Na_2MoO_4 \cdot 2H_2O$ per liter). The salinity was adjusted to 32 ppt and the pH to 7.5 After filtration through a 0.45 μm membrane filter (pre-rinsed w/H_2O), 1 ml/1 sterilized Fe-EDTA solution (33.05 μg Fe/ml as $FeCl_3$+ 300 μg/ml Na_2EDTA) was added aseptically. All three marine species were grown at 17-18°C and 450-550 ft-c continuous illumination. Dunaliella and Porphyridium cultures were placed on a reciprocating shaker (100 oscillations/min), while the diatoms Skeletonema and Thalassiosira were kept static and hand-swirled once/day.

Since we were interested in examining the response of several species of algae to a number of chloro-organic compounds, batch culture was the method of choice. This method, in comparison with continuous-culture assay, provides a quick and relatively simple method for establishing the relative toxicity of pollutants as well as the range of toxicity of each pollutant.

The algae were grown in 100 ml aliquots in sterilized 250 ml Erlenmeyer flasks capped by Bellco "Kap-Uts" covers. To initiate an experiment, sufficient inoculum was added to sterile growth medium to give the following initial cell concentrations: 1×10^3 cells/ml for Dunaliella and Skeletonema; 1×10^4 cells/ml for Porphyridium, Thalassiosira, Scenedesmus, and Selenastrum; and 5×10^4 cells/ml for Microcystis. The test chemicals, dissolved in absolute ethanol, were then added to the culture aseptically so that the final concentration of each chemical along with its solvent ethanol in the medium was 0.1 ppm and 0.01%, respectively. The concentration of the chemicals to be tested, 0.1 ppm, is considerably higher than expected to be present in the environment (17). If a given chemical at a concentration substantially higher than that occurring in the environment proves to have no

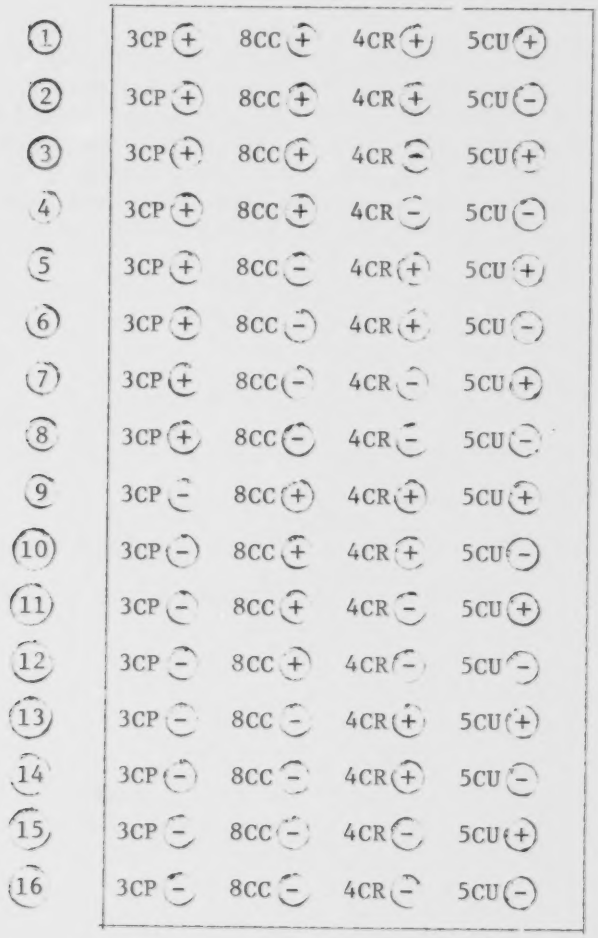
effect on the algae, the safety of the chemical(s) to the algae will have been established. The ethanol at the concentrations used was shown to have no effect on the growth of any of the algae except for Microcystis. In non-axenic cultures of Microcystis, 0.01% ethanol severely repressed algal growth. If the ethanol was eliminated, the algae exhibited normal growth. Therefore, in Microcystis experiments, 100 µl of the selected chemical(s) in ethanol was added to each sterile flask which was then capped by a Bellco "Kap-Uts" cover. The flasks were placed on a warm surface (30°C) overnight so that the ethanol evaporated and diffused out of the flasks. The following day, 100 ml of modified Gorham's medium was added to each flask. The flasks were placed on a reciprocating shaker (100 oscillations/min) for 8 hr at 22°C to dissolve the chemical(s). Tests with one of the chloro-organic chemicals, 5-chloro-uracil-^{14}C (carbon uniformly labeled), showed that 97.7% of the dried chemical redissolved in Gorham's medium in 1 hr and 99.3% by 4 hr.

The growth of the algae was measured every two to three days following treatment (except in the case of Skeletonema) by measuring the absorbance at 650 nm in a Cary spectrophotometer. The experiments were continued at least until the stationary plateau had been reached (i.e., the yield), which took 10-13 days in most experiments and up to 40 days in the Porphyridium experiments. Calibration curves relating absorbance at 650 nm and the number of cells, using a Model B Coulter Counter® and the Cary spectrophotometer were prepared (Figures A1-A8). Approximately 20% of all readings were verified by counting directly the number of cells with a Coulter Counter Model B or an American Optical Bright-line hemacytometer and comparing with the calibration curves (Table A15).

EXPERIMENTAL DESIGN

By means of a factorial experiment (6), the effects of a number of different combinations can be investigated simultaneously. The decision to use the factorial design was based on the desire to gain as much information as possible on the interrelationships between the chemicals and their effects on algal growth, and still work within the time and resource constraints imposed by the project. To study the effects of all seven chemicals in all combinations on a single alga would require 2^7 or 128 flasks with no repetitions. Instead, it was decided to use for each alga four protocols of 16 flasks each, and also repeat some of the chemical combinations two and three times in the four protocols. Each of the four protocols consisted of 15 treatments plus a control. In another protocol, a different combination of four chemicals was tested. Not all possible combinations of four chemicals were tested, nor was any combination involving more than four chemicals tested. The concentration of each chemical was 0.1 ppm. When a mixture was used, the total concentration of chloro-organic chemicals was 0.2, 0.3, or 0.4 ppm depending if 2, 3, or 4 chemicals were used in combination.

A typical experimental protocol is shown in Figure 2, where ⊕ means the chemical is present and ⊖ means it is absent, e.g. combination ⑤ has chemicals: 3CP, 4CR, and 5CU.


1 3CP + 8CC + 4CR + 5CU +
2 3CP + 8CC + 4CR + 5CU -
3 3CP + 8CC + 4CR - 5CU +
4 3CP + 8CC + 4CR - 5CU -
5 3CP + 8CC - 4CR + 5CU +
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12 3CP - 8CC + 4CR - 5CU -
13 3CP - 8CC - 4CR + 5CU +
14 3CP - 8CC - 4CR + 5CU -
15 3CP - 8CC - 4CR - 5CU +
16 3CP - 8CC - 4CR - 5CU -

Figure 2. Typical protocol for a four-chemical factorial experiment.

(1) -- (16) represent the culture flask number in a given experiment. Number 16 is the control.

3CP = 3-chlorophenol
4CR = 4-chlororesorcinol
5CU = 5-chlorouridine
8CC = 8-chlorocaffeine

ANALYSIS OF GROWTH DATA

When a combination of chemicals is added to a culture of algae, the overall growth effect model of a combination is the sum of each chemical taken alone plus any interactions among the chemicals that may occur. For the combination of chemicals A and B, the growth effect would be the algebraic sum of the effect of A alone plus the effect of B alone, plus any interaction effect of A plus B together beyond the effects of either alone. Thus, the overall yield, $Y_{A,B}$, will contain the following factorial components:

$$Y_{A,B} = \mu + k'_A + k'_B + K'_{AB} + \text{error factor},$$

where μ = average yield of all flasks in a protocol.

k'_A = main effect, due to chemical A

k'_B = main effect, due to chemical B

k'_{AB} = interaction effect, due to chemicals A plus B.

Since the error factor is not known, an estimate of $Y_{A,B}$ is obtained denoted as $\hat{Y}_{A,B}$. Thus:

$$\hat{Y}_{A,B} = \mu + k'_A + k'_B + k'_{AB}.$$

The main effects and interactions (k') for each component of Y were obtained from the yield (k) of each treatment using computer analysis and the methods of Yates (6). Once the k' values were obtained, they were tested for significance using the F test.

In the analysis of variance using the F test, the error variance was calculated assuming that three and four factor interactions were negligible (6) and could be used as an estimate of the population variance. Thus, for a particular protocol, the effect means (k') for all three and four factor interactions of a protocol were pooled and the error variance calculated. The F test was then used to determine the significance of each one, two, and any large three and four factor interactions. If the F test showed a chemical or interaction k' value to be significant, the overall growth constant, $\hat{Y}$, for that treatment was calculated and tested for significant difference from the control using the t-test.

UPTAKE OF CHLORO-ORGANIC CHEMICALS BY PHYTOPLANKTON

The chemicals used for these studies were ^{14}C-labeled 3-chlorobenzoic acid, 5-chlorouracil, and 4-chlororesorcinol. The two chemicals 5-chlorouracil and 4-chlororesorcinol were chosen because of their toxic potential (27), whereas, 3-chlorobenzoic acid was selected since it was readily available in

radioactive-labeled form. Four species were used in this study, two of which were marine (Dunaliella tertiolecta and Skeletonema costatum) and two fresh-water (Selenastrum capricornutum and Scenedesmus obliquus). Scenedesmus and Selenastrum were grown in the medium described earlier. Since the modified Burkholder's medium used in the growth studies did not give sufficient biomass needed for the uptake studies, a fortified f/2 medium of Guillard and Ryther (14) was substituted. The composition of the medium is as follows: $NaNO_3$ (75 mg), $NaH_2PO_4 \cdot H_2O$ (5 mg), $NaSiO_3 \cdot 9H_2O$ (15-30 mg), $Na_2 \cdot$EDTA (4.35 mg), $FeCl_3 \cdot 6H_2O$ (3.15 mg), $CuSO_4 \cdot 5H_2O$ (0.01 mg), $ZnSO_4 \cdot 7H_2O$ (0.022 mg), $CoCl_2 \cdot 6H_2O$ (0.01 mg), $MnCl_2 \cdot 4H_2O$ (0.18 mg), $Na_2MoO_4 \cdot 2H_2O$ (0.006 mg), thiamine·HCl (0.1 mg), biotin (0.5 ug), vitamin B_{12} (0.5 ug) in one liter of aged sea-water.

The above medium was filtered to remove any suspended material, buffered with 5 mM glycyglycine, adjusted to pH 7.5, autoclaved, and allowed to stand over night at room temperature. The following day, the previously described autoclaved major nutrients and trace elements were added aseptically. A stock solution of ^{14}C-chlorobenzoic acid, ^{14}C-chlorouracil, or ^{14}C-chlororesorcinol in ethanol or methanol was added to a suspension of exponentially growing cultures with a cell density of 10^5 cells/ml for Scenedesmus and Selenastrum and 10^6 cells/ml for Skeletonema and Dunaliella. The final concentration of the ^{14}C-chemical in the cell suspension was 1 ppm. The concentration of methanol or ethanol did not exceed 0.08%. The rationale for using a higher concentration of the chemical in the uptake studies was to facilitate detection of the chemical because its uptake by the cells was low at 0.1 ppm and our previous studies showed 3-chlorobenzoic acid, 5-chlorouracil, and 4-chloro-resorcinol to be non-toxic to marine algae at a concentration of 1 ppm (27). The cultures were agitated on a rotary shaker to keep the cells in suspension. For determining uptake of chlorobenzoic acid, aliquots of cell suspension were withdrawn at 0, 1, 2, 4, 7, 24, and 48 hr and filtered through a 0.45 µm cellulose acetate Millipore™ membrane filter. The filter and the cells were rinsed consecutively with 5 ml, 10 ml, and 5 ml of fresh growth medium. The filter containing the algae was then transferred to scintillation fluid and counted for ^{14}C in a Packard Tri-Carb liquid scintillation counter.

In order to determine if the membrane filter retained 3-chlorobenzoic acid, an aliquot of ^{14}C-chlorobenzoic acid solution without the algae was passed through the membrane filter which was then rinsed consecutively with 5 ml, 10 ml, and 5 ml of the fresh medium. The filter was then transferred to a scintillation vial and counted for radioactivity. Prior to starting the uptake studies, a quench curve was established for the different species. The amount of ^{14}C associated with the cells was corrected for ^{14}C retention (i.e. cpm) by the filter and for cellular quenching.

The procedure used to determine the uptake of ^{14}C-chlorouracil was similar to 3-chlorobenzoic acid-UL-^{14}C. Due to the high retention of 5-chlorouracil by the cellulose acetate Millipore™ membrane filter, this filter was replaced by a Gelman™ DM-450 filter (0.45 µm pore diameter), a copolymer of acrylonitrile and polyvinyl chloride, which showed a very low retention for the chemical. The amount of ^{14}C uptake was corrected for this filter retention as well as for quenching.

In uptake studies studies with ^{14}C-chlororesorcinol, it was necessary, however, to use centrifugation as a means of collecting and washing cells since all the membrane filters tested retained a significant amount of the chemical, which interfered with the measurement of the chemical's uptake by the cells. At each time point, duplicate 5-ml cell suspensions were centrifuged for 5 minutes at 2500 rpm. The supernatant was removed and the pellet washed twice by suspending it in 10 ml of the fresh medium without the chemical and then centrifuging the suspension. After the washings, the cells were extracted with 5 ml of ethanol and then transferred to a scintillation vial for counting for radioactivity. Separate controls indicated that the adsorption of the chemical to the glassware was too low to be detected.

METABOLISM OF 4-CHLORORESORCINOL BY PHYTOPLANKTON

In order to obtain sufficient cellular material for studying metabolism of chlororesorcinol by Skeletonema and Selenastrum, the algae were cultured in Fernbach flasks in 2 liters of the appropriate growth medium. ^{14}C-Chlororesorcinol was added to the cell suspension when it reached a density of approximately 10^6 cells/ml. After it was determined that the cells contained sufficient radioactivity for chromatographic purposes, the cells were separated from the medium by centrifugation. The pellet was resuspended in the growth medium, centrifuged, the supernatant discarded. This pellet was then extracted twice with methanol. The methanol extract was concentrated under vacuum and spotted on thin-layer silica-gel and cellulose plates. The plates were developed in the following systems:

Cellulose plates

1) water-saturated toluene:acetic acid (4:1)
2) ethanol:water:ammonium hydroxide (16:3:1)

Silica-gel plates

1) chloroform:ethyl acetate:acetic acid (100:5:3)
2) toluene:methanol:acetic acid (45:8:4)

Authentic ^{14}C-chlororesorcinol was cochromatographed for comparison with the unknowns. The plates were scanned for radioactivity on a Nuclear Chicago Actigraph III.

The medium containing the labeled chemical but no algae was acidified to pH 2 and extracted with ethyl ether. The ether extract was counted for ^{14}C, concentrated under vacuum, and chromatographed as described above.

PHOTODEGRADATION OF 4-CHLORORESORCINOL

An aqueous solution of 4-chlororesorcinol was irradiated with a 450-Watt Hanovia medium pressure mercury lamp fitted with a Pyrex 7740 filter which excluded light of wavelength less than 280 nm. As there is no significant amount of radiation in sunlight with a wavelength less than 280 nm, it is

assumed that the photodegradation of the chemical in aqueous solution in these studies would be similar to that occurring in the sunlight. The irradiation was carried out in a photochemical reactor (Ace Glass Company), which consists of a jacketed borosilicate glass vessel and is equipped with a side arm for withdrawing samples. A double-walled water-cooled quartz well, housing the light source and filter, is fitted into the vessel and is immersed in the solution to be irradiated. Aliquots of photolyzed solution were withdrawn at appropriate intervals and analyzed for chlororesorcinol by high-pressure liquid chromatography. The instrument and column used were the same as those described for the purification of 5-chlorouracil. A solvent system consisting of acetonitrile:5% glacial acetic acid in water (50:50 v/v) was used to analyze the chemical. The retention volume of chlororesorcinol on the HPLC column was 3.8 ml. Chlororesorcinol was detected by its absorbance at 283 nm.

SECTION 5

RESULTS

EFFECTS OF CHLORO-ORGANIC CHEMICALS ON THE GROWTH OF PHYTOPLANKTON

It is quite likely that algae in the bodies of water receiving chlorinated waste-waters will be exposed to several chloro-organic chemicals simultaneously. As a result, there may be either synergistic or antagonistic effects produced by the interaction of these chemicals. Individually, a given chemical may not be toxic to certain species. However, the response of a species to a particular chemical may be altered by the presence of another chemical(s). Therefore, in evaluating the effects of chloro-organic chemicals, the interaction among various chemicals should also be considered. Interaction among certain organic pollutants in marine algae has been recently reported by Mosser *et al*. (22). They observed that DDT counteracted the toxicity of PCB's in a marine diatom, whereas DDE and PCB's acted synergistically in inhibiting the growth of the alga. In these studies, we examined the effects of the chemicals alone, as well as in combination with other chloro-organic chemicals to determine if the chemicals interact with each other.

It is well known that phytoplanktonic species vary greatly in their response to pollutants. Dunstan (8) recently reported that different phylogenetic groups of marine phytoplankton and species within these groups varied considerably in their response to effluent from the same sewage treatment plant. Since the results based on the effects on one or two species can be misleading, we examined the effects of the chemicals in several algal species.

Yield data of algae with selected chemicals are shown in Tables A1-A14. Of the 278 different treatments representing single chemicals and their combinations with 7 algal species, only 21 were found to either significantly stimulate or inhibit algal growth. Of these 21 treatments, the effects due to 9 treatments, when tested in other protocols, were found to be non-significant. Chloroguanine may possibly have an effect on *Thalassiosira*. It reduced the growth by 6% in one protocol and by 8.5% in another. The effects of the remaining 11 significant treatments were tested only once, with six treatments being stimulatory and five being inhibitory.

Because of these inconsistent results, most if not all of the significant results must be treated as being inconclusive. As a result, it is concluded that these chemicals at 0.1 ppm alone or in combinations of up to four chemicals had little effect, if any, on the yield of algae tested. It is

suggested, however, that further selected testing should be carried out to substantiate one way or another the effects of some of these chemicals on algal growth.

EFFECT OF NITRATE CONCENTRATION ON THE TOXICITY OF THE TEST CHEMICALS

Environmental factors such as temperature, salinity, light, pH and nutrients influence the metabolic activity of algae. It is expected that the response of the organisms to toxicants may vary with changes in these factors. An organism may become more susceptible to outside stress under environmental conditions which do not favor its optimal growth (3, 11, 19,20,21). Hannan and Patouillet (16) observed that the toxicity of mercury to algae increased with decreasing nutrient concentrations. Fisher et al. (11) reported that the growth of the diatom Thalassiosira was unaffected by PCBs in high nitrate media, but was substantially reduced in the media containing lower nitrate levels.

In order to assess the influence of nutrient level on the toxicity of the chloro-organic chemicals, the algae were grown in the media containing the test chemical(s) and varying amounts of nitrate. Two nitrate levels were examined: (i) that specified in the Algal Assay Procedure, EPA (1971) and (ii) 10% of this level. These studies included the effects of three chloro-organic chemicals on Selenastrum and Scenedesmus.

As shown in Figures 3 and 4, nitrate deprivation does not appear to have any influence on the effects of the chloro-organic chemicals.

UPTAKE OF CHLORO-ORGANIC COMPOUNDS BY PHYTOPLANKTON

These studies were done using ^{14}C-labelled chemicals. The chemicals used for these studies were 3-chlorobenzoic acid, 4-chlororesorcinol, and 5-chlorouracil. The species included in these studies were Dunaliella tertiolecta, Skeletonema costatum (both marine), Selenastrum capricornutum and Scenedesmus obliquus (both fresh-water).

The uptake studies were carried out at an initial concentration of 1 ppm of the test chemical in the medium and a cell density of 10^5 - 10^6 cells/ml. The rationale for using a higher concentration of the chemicals and a higher cell density compared to the growth experiments was to facilitate detection of the chemicals if their uptake by the organisms was low.

4-Chlororesorcinol: The uptake of ^{14}C-4-chlororesorcinol by all four species was rapid (Table 1). In the cases of Scenedesmus, Selenastrum, and Skeletonema, uptake of the chemical reached its maximum within one hour of treatment, whereas the maximum uptake in Dunaliella was observed 24 hours after treatment. The four species varied in their ability to accumulate chlororesorcinol from the medium. They accumulated the chemical in the following order: Skeletonema > Dunaliella > Selenastrum > Scenedesmus.

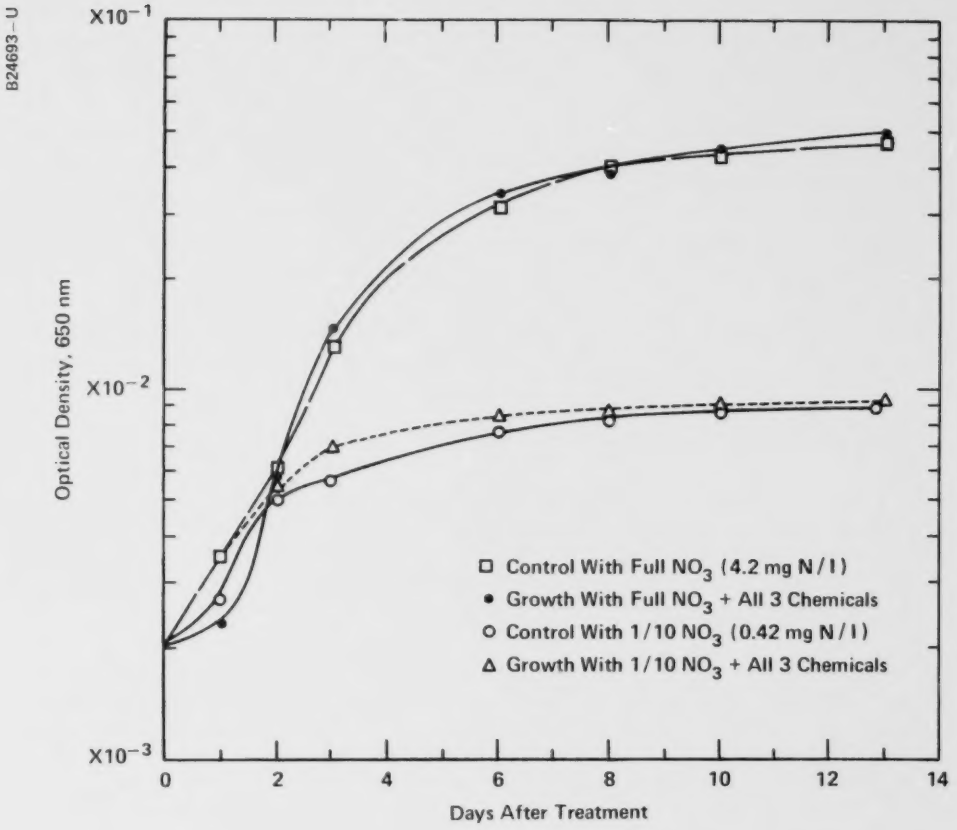

Figure 3. Effect of 3-chlorophenol, 4-chlororesorcinol and 5-chlorouracil at 0.1 ppm on the growth of Scenedesmus obliquus at two levels of nitrate concentration.

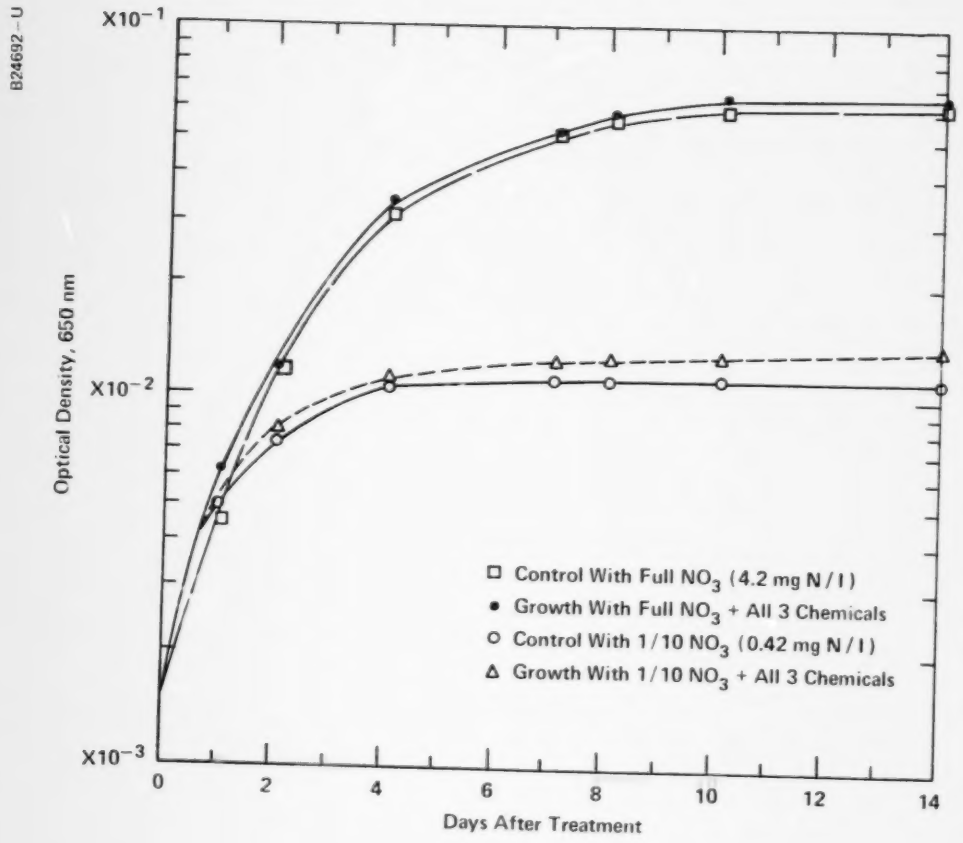

Figure 4. Effect of 3-chlorophenol, 4-chlororesorcinol, and 5-chlorouracil at 0.1 ppm on the growth of Selenastrum capricornutum as a function of two levels of nitrate concentration.

TABLE 1. UPTAKE OF ^{14}C-CHLORORESORCINOL BY PHYTOPLANKTON

Hours after treatment	Concentration of ^{14}C-Residue in the cells-ppm (expressed as chlororesorcinol equivalent) [a]			
	Scenedesmus obliquus	Selenastrum capricornutum	Skeletonema costatum	Dunaliella tertiolecta
0	0.074[b] (73.7)[c]	0.102 (101.6)	0.119 (118.6)	0.083 (82.9)
1	0.039 (38.7)	0.107 (107.1)	0.294 (293.7)	0.074 (73.7)
2	0.048 (47.1)	--- ---	0.277 (277.4)	--- ---
4	0.048 (47.9)	--- ---	0.296 (295.7)	0.097 (97.3)
7½	0.040 (39.7)	0.151 (151.3)	0.309 (308.9)	0.130 (130.2)
24	0.036 (36.1)	0.093 (93.0)	0.206 (206.3)	0.182 (182.3)

a/ Calculated from the specific activity of ^{14}C-chlororesorcinol.

b/ Each value represents the mean of 4 replications.

c/ Values in parentheses indicate bioconcentration factors.

TABLE 2. UPTAKE OF ^{14}C-CHLOROURACIL BY PHOTOPLANKTON

Hours after treatment	Concentration of ^{14}C-Residue in the cells-ppm[a/] (expressed as chlorouracil equivalent)			
	Scenedesmus obliquus	Selenastrum capricornutum	Skeletonema costatum	Dunaliella tertiolecta
0	0.011[b/] (11)[c/]	0.000 (0)	0.034 (33.7)	0.000 (0)
1	0.012 (12)	0.000 (0)	0.025 (24.9)	0.000 (0)
2	---	---	0.026 (25.7)	0.000 (0)
4	0.049 (49)	0.000 (0)	0.025 (25.1)	0.000 (0)
8	0.067 (67)	0.000 (0)	0.046 (45.6)	0.000 (0)
24	0.018 (48)	0.000 (0)	0.042 (42.3)	0.000 (0)
48	0.050 (50)	0.000 (0)	--- ---	--- -

a/ Calculated from the specific activity of ^{14}C-chlorouracil.

b/ Each value represents the mean of 4 replications.

c/ Values in parentheses indicate bioconcentration factors.

TABLE 3. UPTAKE OF ^{14}C-CHLOROBENZOIC ACID BY PHYTOPLANKTON

Hours after treatment	Concentration of ^{14}C-Residue in the cells-ppm (expressed as chlorobenzoic equivalents) [a/]			
	Scenedesmus obliquus	Selenastrum capricornutum	Skeletonema costatum	Dunaliella tertiolecta
0	0.000[b/] (0)[c/]	0.000 (0)	0.007 (7.0)	0.004 (3.8)
1	0.000 (0)	0.000 (0)	0.000 (0)	0.000 (0)
2	0.000 (0)	0.000 (0)	0.000 (0)	0.000 (0)
4	0.000 (0)	0.000 (0)	0.000 (0)	0.000 (0)
7	0.000 (0)	0.000 (0)	0.000 (0)	0.000 (0)
24	0.000 (0)	0.000 (0)	0.000 (0)	0.000 (0)
48	0.000 (0)	0.000 (0)	0.000 (0)	0.000 (0)

a/ Calculated from the specific activity of ^{14}C-chlorobenzoic acid.

b/ Each value represents the mean of 4 replications.

c/ Values in parentheses indicate bioconcentration factors.

5-Chlorouracil: Skeletonema and Scenedesmus appeared to remove small amounts of chlorouracil from the medium, whereas Dunaliella and Selenastrum showed no capacity for taking up the chemical (Table 2). In the case of Skeletonema, maximum uptake of chlorouracil was noticed within one hour of treatment. However, in Scenedesmus maximum uptake was noticed 8 hours after treatment.

3-Chlorobenzoic Acid: None of the species appeared to take up the chemical from the medium (Table 3).

Although the algae accumulated both chlororesorcinol and chlorouracil from the medium, bioaccumulation of chlororesorcinol was greater than that of chlorouracil. The concentration of ^{14}C-chlororesorcinol and its degradation products in the algae at equilibration ranged from 38 to 308 times the concentration in the medium. In the case of algae treated with ^{14}C-chlorouracil, the bioaccumulation ranged from about 26 to 67. The bioaccumulation of chlororesorcinol and chlorouracil by phytoplankton is considerably lower than that reported for chlorinated hydrocarbons (25,26). The lower uptake of the two chemicals may be explained by their relatively high water-solubility (lower lipophilicity)and their low pKa's (4,23,24). Since at the pH of the medium used the chemicals are expected to be present mostly in an ionized form, they are less likely to partition from the water into the algal cells.

METABOLISM OF 4-CHLORORESORCINOL BY PHYTOPLANKTON

For metabolism studies, only those chemical-algal systems were investigated which showed sufficient uptake of the ^{14}C-chemical so that the radioactivity in the cells could be characterized by chromatographic procedures. For this reason, we examined only the metabolism of ^{14}C-chlororesorcinol in two species, Skeletonema and Selenastrum. Although ^{14}C-chlorouracil was taken up by the algae, the uptake was not sufficient to permit the characterization of ^{14}C-material in the cells. To obtain sufficient amounts of ^{14}C-materials for chromatographic analysis, the metabolism studies were done with algae grown in large batch-cultures.

In our initial studies on the metabolism of ^{14}C-chlororesorcinol by Skeletonema, the cells were incubated with the chemical for 10 days. The cells were then extracted and the extract chromatographed on thin-layer silica-gel and cellulose plates.

Thin-layer chromatography of the cell extract on the cellulose plates showed 3-4 major peaks. However, the peaks were poorly resolved and showed considerable tailing. Because silica-gel plates showed better resolution they were used for chromatography in the subsequent studies.

Table 4 shows that Skeletonema either extensively metabolized the 4-chlororesorcinol in vivo or took up its photodegradation products from the medium. In order to distinguish between these possibilities, metabolism of ^{14}C-chlororesorcinol was examined in Skeletonema incubated with the chemical in light as well as in the dark. In these studies the algae were incubated with the ^{14}C-chemical for 24 hours. Similar experiments were conducted to

study the metabolism of ^{14}C-chlororesorcinol by Selenastrum. We also investigated if chlororesorcinol was degraded by light in the culture medium without the algae.

TABLE 4. DISTRIBUTION OF ^{14}C-CHLORORESORCINOL AND ITS DEGRADATION PRODUCTS IN SKELETONEMA INCUBATED WITH THE CHEMICAL FOR 10 DAYS

Compound	% of ^{14}C in the methanol extract	R_f 1/
Unknown	66	0.00
4-chlororesorcinol	8	0.31
Unknown	4	0.48
Unknown	3	0.66
Unknown	19	0.86

1/ Silica-gel; chloroform:ethyl acetate:acetic acid (100:5:3)

Thin-layer chromatography of the methanol extract of Skeletonema or Selenastrum incubated with ^{14}C-chlororesorcinol for 24 hours in the light showed the presence of two ^{14}C-materials (Table 5). One ^{14}C-compound co-chromatographed with authentic chlororesorcinol while the other radioactive compound(s) remained near the origin indicating that it was more polar than chlororesorcinol. All the ^{14}C in the extract from algae incubated with ^{14}C-chlororesorcinol in the dark was present as the parent chemical. Thin-layer chromatographic analysis of the sterile medium incubated with ^{14}C-chlororesorcinol for 24 hours showed the presence of the same two ^{14}C-compounds as those detected in the algae incubated with the ^{14}C-chemical in the light. These results indicate that chlororesorcinol is degraded by light to compound(s) similar to that found in algae. The fact that the chlororesorcinol metabolites were detected only in the algae incubated with the chemical in the light but not in the dark suggests that Skeletonema and Selenastrum metabolized the chemical through a light-driven reaction and/or they had taken up chlororesorcinol photoproducts from the medium. Further studies are needed to distinguish between these possibilities.

PHOTODEGRADATION OF 4-CHLORORESORCINOL

The results of the above experiments showed that chlororesorcinol is degraded by light. These studies were done in a growth chamber under light provided by a mixture of fluorescent lamps. Since this light is a poor source of U.V. light, it does not simulate sunlight. In order to obtain some information on the photodegradation of chlororesorcinol under environmental conditions, we examined the degradation of the chemical under simulated sunlight.

TABLE 5. DISTRIBUTION OF ^{14}C-CHLORORESORCINOL AND ITS DEGRADATION PRODUCTS IN SKELETONEMA AND SELENASTRUM INCUBATED WITH THE CHEMICAL IN THE LIGHT AND DARK

Compound	Skeletonema		Selanastrum		Sterile Medium		R_f Value 1/	
	% of ^{14}C in the Cell Extract		% of ^{14}C in the Cell Extract		% of ^{14}C in the Medium			
	Light	Dark	Light	Dark	Light	Dark	System I	System II
4-chlororesorcinol	65	100	89	100	96	100	0.41	0.43
Unknown	35	--	11	--	4	--	0.00	0.00

1/ Solvent System I: Chloroform:ethyl acetate:acetic acid (100:5:3)
Solvent System II: Toluene:methanol:acetic acid (45:8:4)

It was noticed that 24% of the chemical had degraded within 5 hours of irradiation with a 450-watt medium pressure mercury lamp fitted with a Pyrex 7740 filter (Figure 5). After 24 hours, only 33% of the original chemical was present in the solution. These findings confirm our earlier results that chlororesorcinol is readily degraded by the action of light. Further studies are recommended for characterizing the products resulting from photodegradation of chlororesorcinol.

Since chlororesorcinol readily undergoes photodegradation, one would expect that in bodies of water contaminated with chlororesorcinol, phytoplankton would be exposed to its photodegradation products. In order to have comprehensive information on the interaction of chlororesorcinol with phytoplankton, it is important to assess the toxicity and bioaccumulation of the photodegradation products of the chemical by the organisms.

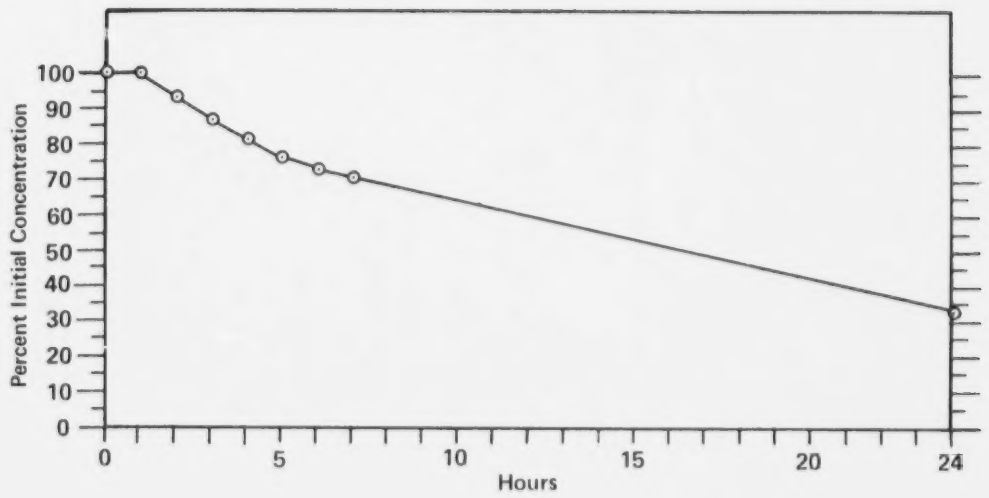

Figure 5. Photodegradation of 4-chlororesorcinol in simulated sunlight.

SECTION 6

DISCUSSION

This study has shown that several stable chloro-organic chemicals produced during chlorination of domestic waste-water do not affect the growth of a number of fresh-water and marine phytoplankton species. The chemicals were ineffective when added alone at a concentration of 0.1 ppm or in combination of 2 to 4 chemicals, indicating a lack of interaction among the chemicals. The findings suggest that at the concentrations tested, the chloro-organic chemicals are not expected to have any significant effect on primary productivity in the aquatic environment.

These findings suggest that phytoplankton are less sensitive than other aquatic organisms to chloro-organic compounds. Gehrs et al. (12) observed that both 5-chlorouracil and 4-chlororesorcinol decreased the hatchability of carp eggs at concentrations as low as 1 ppb. In order to fully assess the effect of the chloro-organic chemicals on aquatic organisms, their toxicity to aquatic invertebrates and fishes should be determined since these organisms are generally much more sensitive to low concentration of chemicals.

The investigation has also demonstrated that chlorouracil and chloro-resorcinol are accumulated by algae although the extent of accumulation is far less than that of chlorinated hydrocarbons. Although both the chemicals appear to have no adverse effect on the phytoplankton themselves at the concentrations tested, their bioaccumulation is potentially important. Chlorouracil and chlororesorcinol accumulated by algae may be transferred to the higher members of the food chain which are more sensitive to low levels of chemical pollutants. On account of the mutagenic potential of chloro-uracil (7), the bioaccumulation of even small amounts of this chemical by algae noticed in our studies is of concern since other members of the food chain may become exposed to this potentially hazardous compound.

The presence of chloro groups is known to make aromatic compounds more resistant to microbial degradation which suggests that chlorine-containing organic compounds introduced via chlorinated waste-water effluents may accumulate in aquatic environments. Since these chemicals are being continuously added to the aquatic ecosystem, their concentration may build up if the chemicals are not readily degraded. It therefore becomes important to determine the environmental persistence of these chemicals. In the event the chemicals are found not to readily degrade, their effect on aquatic organisms should be studied at concentrations higher than those used in these

studies. Furthermore, the chloro-organic chemicals may be converted to other stable products as a result of biological and/or nonbiological transformation in the aquatic environment. To fully assess the effects of chloro-organic chemicals on phytoplankton, we suggest that the effects of their transformation products be determined.

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APPENDIX

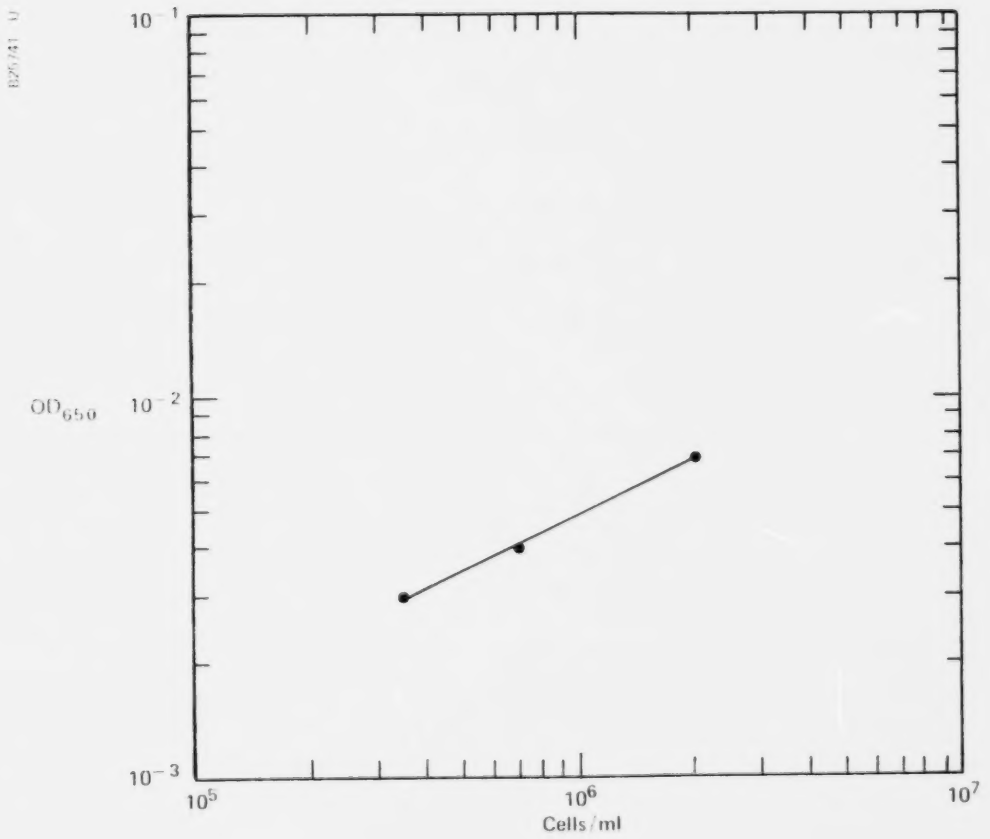

Figure A1. Calibration curve (cell number vs. optical density) for Microcystis aeruginosa

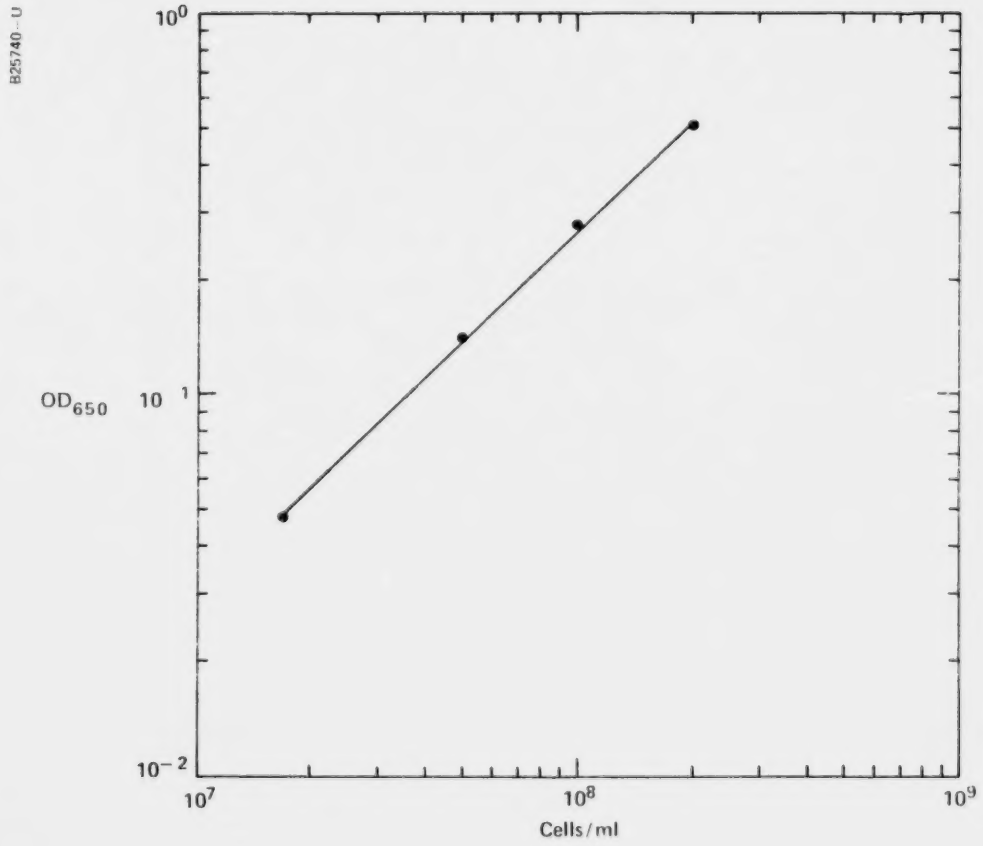

Figure A2. Calibration curve (cell number vs. optical density) for Microcystis aeruginosa.

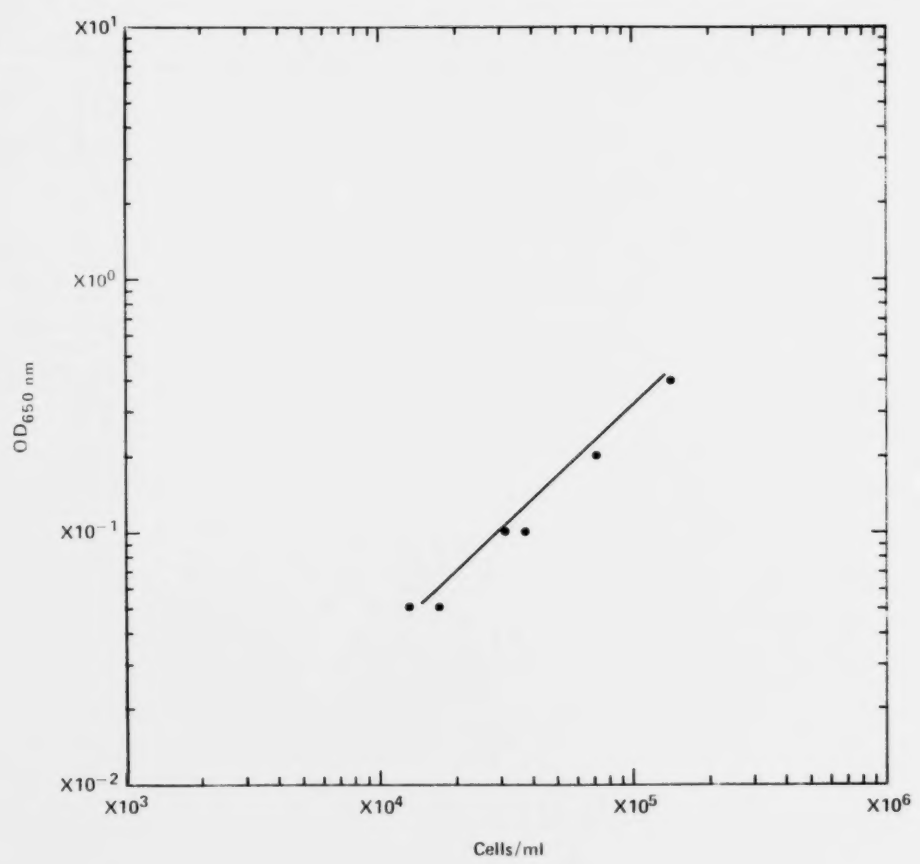

Figure A3. Calibration curve (cell number vs. optical density) for Dunaliella tertiolecta

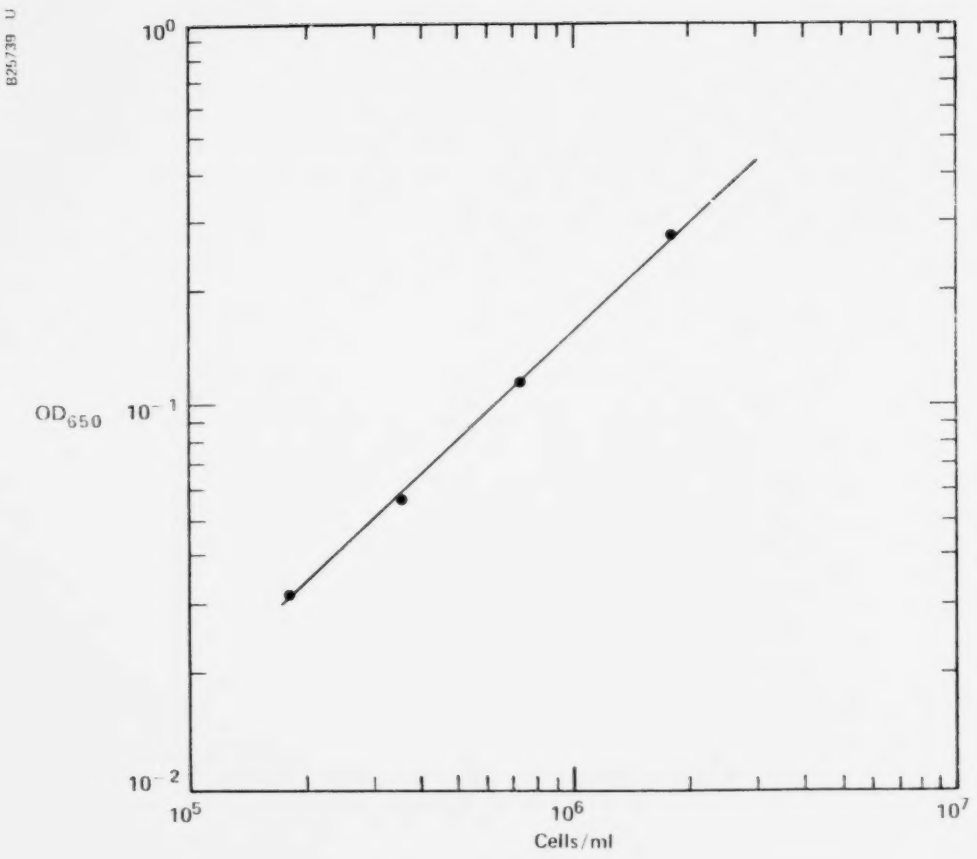

Figure A4. Calibration curve (cell number vs. optical density) for Porphyridium sp.

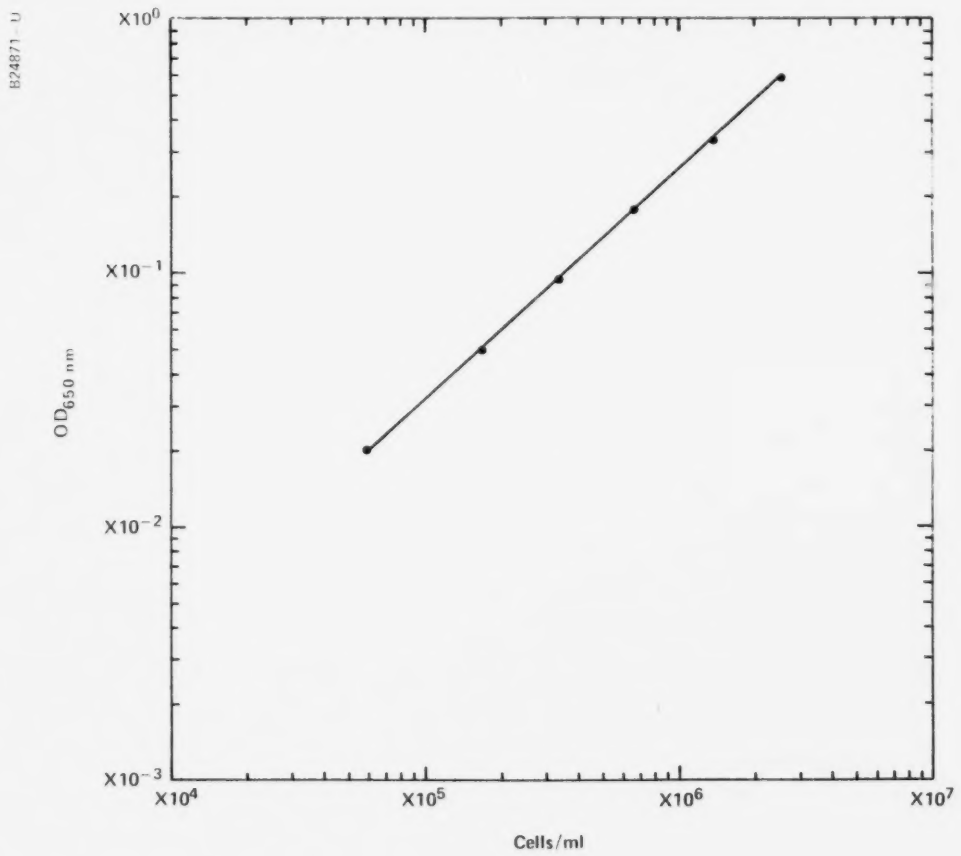

Figure A5. Calibration curve (cell number vs. optical density) for Scenedesmus obliquus.

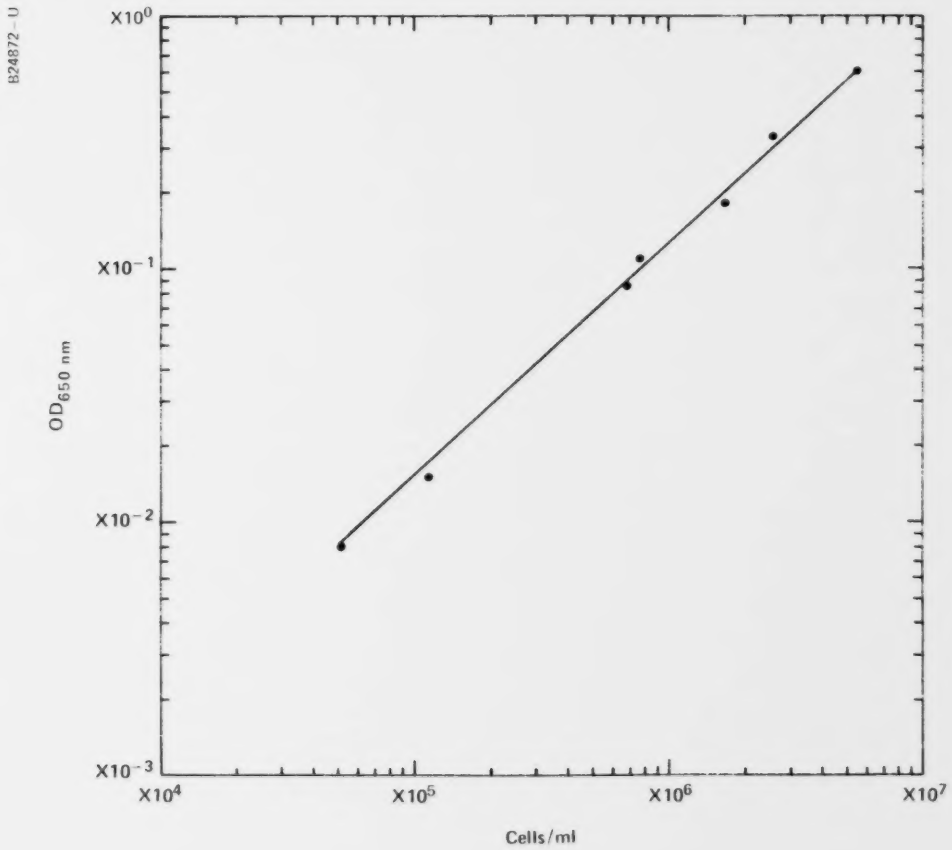

Figure A6. Calibration curve (cell number vs. optical density) for Selenastrum capricornutum.

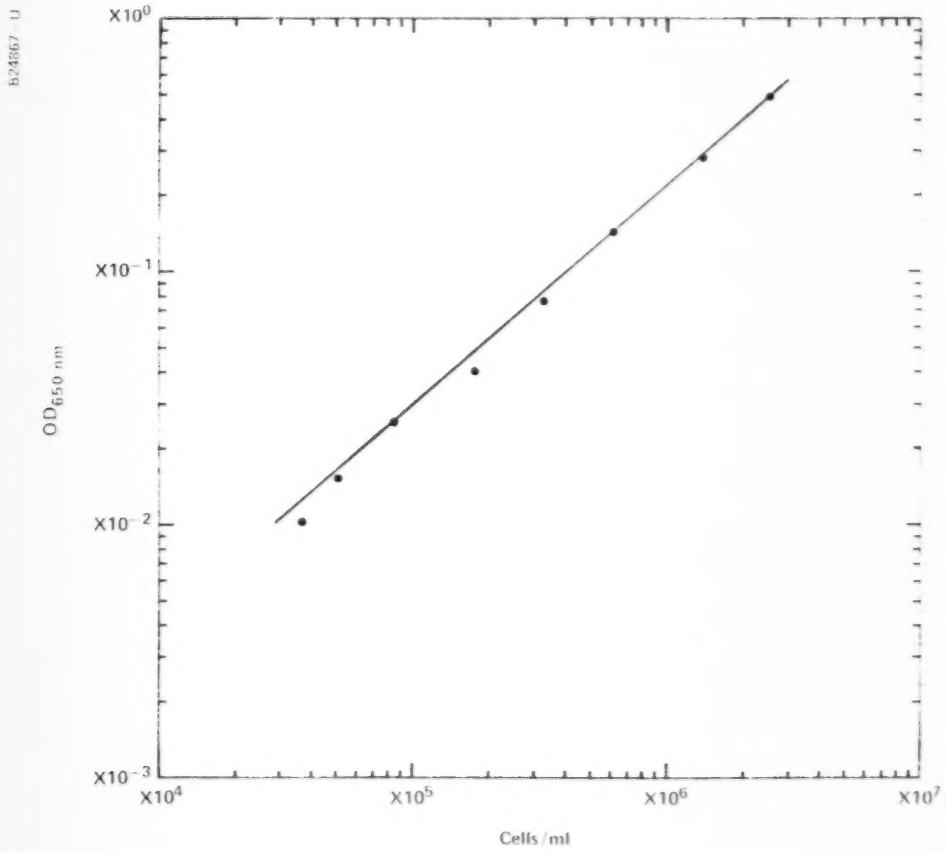

Figure A7. Calibration curve (cell number vs. optical density) for Skeletonema costatum.

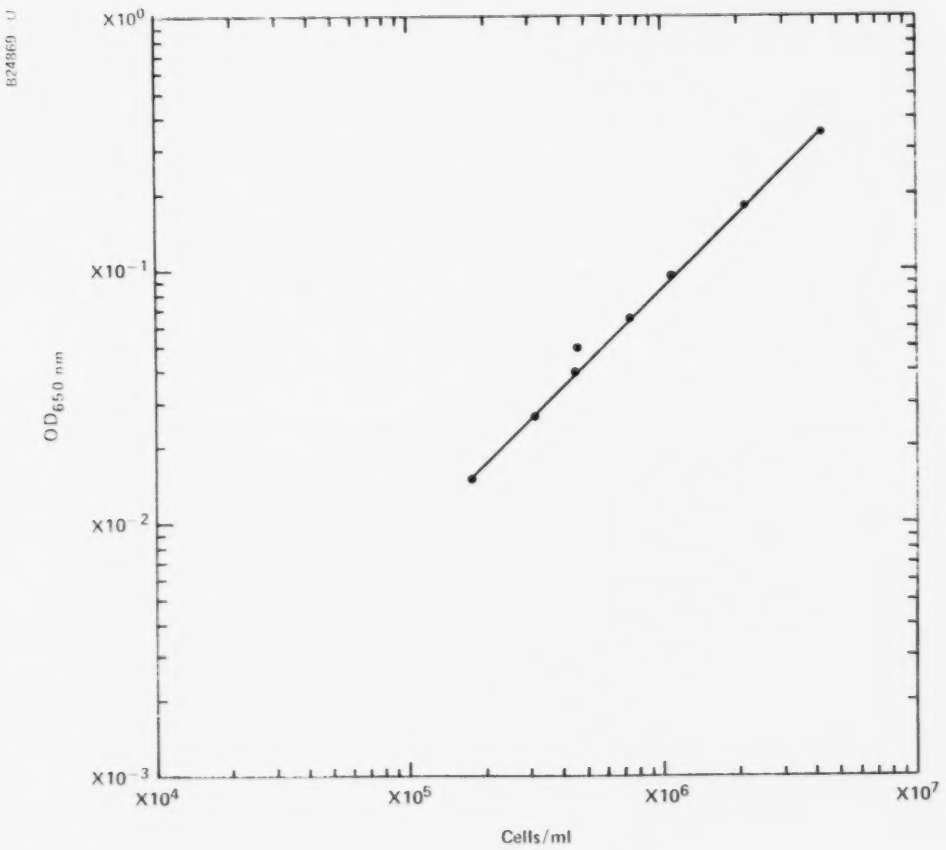

Figure A8. Calibration curve (cell number vs. optical density) for Thalassiosira pseudonana.

TABLE A1. THE EFFECTS OF VARIOUS CHLORO-ORGANIC CHEMICALS AT 0.1 ppm ON THE YIELD OF *DUNALIELLA TERTIOLECTA*

Experiment 1			Experiment 2		
Treatment	Biomass (cells/ml)	Main effects & interactions	Treatment	Biomass (cells/ml)	Main effects & interactions
CR	7.4×10^4	$-.04 \times 10^4$	CC	7.2×10^4	$.00 \times 10^4$
CP	8.2×10^4	$-.09 \times 10^4$	CU	7.6×10^4	$.15 \times 10^4$
CG	7.0×10^4	$-.14 \times 10^4$	CUD	7.2×10^4	$-.15 \times 10^4$
CC	7.4×10^4	$-.21 \times 10^4$	CB	7.4×10^4	$.05 \times 10^4$
CP, CR	7.5×10^4	$-.24 \times 10^4$	CC, CU	7.6×10^4	$-.05 \times 10^4$
CG, CR	7.8×10^4	$.21 \times 10^4$	CC, CUD	7.6×10^4	$-.05 \times 10^4$
CC, CR	7.4×10^4	$.14 \times 10^4$	CB, CC	7.6×10^4	$-.05 \times 10^4$
CG, CP	7.8×10^4	$.24 \times 10^4$	CU, CUD	7.2×10^4	$.00 \times 10^4$
CC, CP	7.5×10^4	$-.12 \times 10^4$	CB, CU	7.4×10^4	$.10 \times 10^4$
CC, CG	7.6×10^4	$.09 \times 10^4$	CB, CUD	7.2×10^4	$.00 \times 10^4$
CG, CP, CR	7.4×10^4	$-.19 \times 10^4$	CC, CU, CUD	7.2×10^4	$-.10 \times 10^4$
CC, CP, CR	7.4×10^4	$.14 \times 10^4$	CB, CC, CU	7.6×10^4	$.00 \times 10^4$
CC, CG, CP	7.0×10^4	$-.21 \times 10^4$	CB, CU, CUD	7.8×10^4	$.25 \times 10^4$
CC, CG, CR	8.0×10^4	$-.16 \times 10^4$	CB, CC, CUD	7.0×10^4	$-.20 \times 10^4$
CC, CG, CP, CR	6.9×10^4	$.04 \times 10^4$	CB, CC, CU, CUD	7.4×10^4	$.05 \times 10^4$
Control	7.8×10^4		Control	7.4×10^4	

* Denotes significance at 5% level of significance.

TABLE A2. THE EFFECTS OF VARIOUS CHLORO-ORGANIC CHEMICALS AT 0.1 ppm ON THE YIELD OF DUNALIELLA TERTIOLECTA

Experiment 3			Experiment 4		
Treatment	Biomass (cells/ml)	Main effects & interactions	Treatment	Biomass (cells/ml)	Main effects & interactions
CR	7.2×10^4	$-.19 \times 10^4$	CR	6.7×10^4	$.05 \times 10^4$
CB	7.2×10^4	$-.33 \times 10^4$	CP	6.9×10^4	$.10 \times 10^4$
CUD	7.6×10^4	$.11 \times 10^4$	CG	7.3×10^4	$.13 \times 10^4$
CP	7.2×10^4	$-.31 \times 10^4$	CU	6.9×10^4	$.23 \times 10^4$
CB, CR	6.5×10^4	$-.16 \times 10^4$	CP, CR	7.0×10^4	$.23 \times 10^4$
CR, CUD	7.2×10^4	$.09 \times 10^4$	CG, CR	6.5×10^4	$.00 \times 10^4$
CP, CR	7.0×10^4	$-.09 \times 10^4$	CR, CU	6.9×10^4	$.10 \times 10^4$
CB, CUD	7.0×10^4	$-.01 \times 10^4$	CG, CP	6.5×10^4	$-.05 \times 10^4$
CB, CP	6.9×10^4	$-.13 \times 10^4$	CP, CU	6.9×10^4	$.05 \times 10^4$
CP, CUD	6.9×10^4	$-.24 \times 10^4$	CG, CU	7.0×10^4	$.13 \times 10^4$
CB, CR, CUD	7.5×10^4	$.11 \times 10^4$	CG, CP, CR	6.9×10^4	$.23 \times 10^4$
CB, CP, CR	6.5×10^4	$-.16 \times 10^4$	CP, CR, CU	6.9×10^4	$-.08 \times 10^4$
CB, CP, CUD	6.9×10^4	$-.06 \times 10^4$	CG, CU, CP	7.0×10^4	$.20 \times 10^4$
CP, CR, CUD	7.2×10^4	$-.06 \times 10^4$	CG, CR, CU	7.0×10^4	$.15 \times 10^4$
CB, CP, CR, CUD	6.1×10^4	$-.33 \times 10^4$	CG, CP, CR, CU	7.6×10^4	$-.08 \times 10^4$
Control	7.0×10^4		Control	6.6×10^4	

* Denotes significance at 5% level of significance.

TABLE A3. THE EFFECTS OF VARIOUS CHLORO-ORGANIC CHEMICALS AT 0.1 ppm ON THE YIELD OF *MICROCYSTIS AERUGINOSA*

Experiment 1			Experiment 2		
Treatment	Biomass (cells/ml)	Main effects & interactions	Treatment	Biomass (cells/ml)	Main effects & interactions
CR	2.4×10^7	$.01 \times 10^7$	C	2.7×10^7	$.08 \times 10^8$
CP	2.4×10^7	$-.13 \times 10^7$	CU	2.2×10^7	$-.11 \times 10^8$
CG	2.7×10^7	$.09 \times 10^7$	CUD	2.8×10^7	$.08 \times 10^8$
CC	2.7×10^7	$-.21 \times 10^7$	CB	2.9×10^7	$.12 \times 10^8$
CP, CR	2.3×10^7	$.29 \times 10^7$*	CC, CU	2.6×10^7	$.02 \times 10^8$
CG, CR	2.7×10^7	$.16 \times 10^7$	CC, CUD	2.6×10^7	$-.02 \times 10^8$
CC, CR	2.5×10^7	$.26 \times 10^7$	CB, CC	2.7×10^7	$-.01 \times 10^8$
CG, CP	2.7×10^7	$.31 \times 10^7$*	CU, CUD	2.7×10^7	$.07 \times 10^8$
CC, CP	1.9×10^7	$.11 \times 10^7$	CB, CU	2.7×10^7	$.01 \times 10^8$
CC, CG	2.3×10^7	$.01 \times 10^7$	CD, CUD	2.5×10^7	$-.08 \times 10^8$
CG, CP, CR	2.7×10^7	$-.11 \times 10^7$	CC, CU, CUD	2.7×10^7	$-.11 \times 10^8$
CC, CP, CR	2.5×10^7	$.09 \times 10^7$	CB, CC, CU	2.7×10^7	$-.09 \times 10^8$
CC, CG, CP	2.3×10^7	$.06 \times 10^7$	CB, CU, CUD	2.7×10^7	$-.07 \times 10^8$
CC, CG, CR	2.3×10^7	$.09 \times 10^7$	CB, CC, CUD	3.0×10^7	$.19 \times 10^8$
CC, CG, CP, CR	3.0×10^7	$.09 \times 10^7$	CB, CC, CU, CUD	2.7×10^7	$-.07 \times 10^8$
Control	3.3×10^7		Control	2.0×10^7	

* Denotes significance at 5% level of significance.

TABLE A4. THE EFFECTS OF VARIOUS CHLORO-ORGANIC CHEMICALS AT 0.1 ppm ON THE YIELD OF MICROCYSTIS AERUGINOSA

Experiment 3			Experiment 4		
Treatment	Biomass (cells/ml)	Main effects & interactions	Treatment	Biomass (cells/ml)	Main effects & interactions
CR	2.1×10^7	$.13 \times 10^7$	CR	3.4×10^7	$.16 \times 10^7$
CP	1.8×10^7	$-.05 \times 10^7$	CB	2.9×10^7	$.39 \times 10^7$
CG	2.2×10^7	$.13 \times 10^7$	CUD	3.0×10^7	$-.21 \times 10^7$
CU	2.1×10^7	$-.08 \times 10^7$	CP	2.8×10^7	$-.09 \times 10^7$
CP, CR	2.2×10^7	$.13 \times 10^7$	CB, CR	3.9×10^7	$.19 \times 10^7$
CG, CR	2.0×10^7	$.05 \times 10^7$	CR, CUD	3.0×10^7	$-.26 \times 10^7$
CR, CU	1.8×10^7	$-.05 \times 10^7$	CP, CR	3.4×10^7	$-.09 \times 10^7$
CG, CP	1.9×10^7	$.03 \times 10^7$	CB, CUD	3.4×10^7	$.16 \times 10^7$
CP, CU	1.8×10^7	$-.03 \times 10^7$	CB, CP	3.5×10^7	$.19 \times 10^7$
CG, CU	1.9×10^7	$.05 \times 10^7$	CP, CUD	3.1×10^7	$-.01 \times 10^7$
CG, CP, CR	2.3×10^7	$-.05 \times 10^7$	CB, CR, CUD	3.4×10^7	$.06 \times 10^7$
CP, CR, CU	1.9×10^7	$-.10 \times 10^7$	CB, CP, CR	3.6×10^7	$-.06 \times 10^7$
CG, CP, CU	2.0×10^7	$.00 \times 10^7$	CB, CP, CUD	3.3×10^7	$-.04 \times 10^7$
CG, CR, CU	2.3×10^7	$.13 \times 10^7$	CP, CR, CUD	2.4×10^7	$-.01 \times 10^7$
CG, CP, CR, CU	2.1×10^7	$-.13 \times 10^7$	CB, CP, CR, CUD	3.6×10^7	$.31 \times 10^7$
Control	2.0×10^7		Control	3.4×10^7	

* Denotes significance at 5% level of significance.

TABLE A5. THE EFFECTS OF VARIOUS CHLORO-ORGANIC CHEMICALS AT 0.1 ppm ON THE YIELD OF PORPHYRIDIUM SP.

Experiment 1			Experiment 2		
Treatment	Biomass (cells/ml)	Main effects & interactions	Treatment	Biomass (cells/ml)	Main effects & interactions
CR	3.7×10^5	$-.44 \times 10^5$	CC	1.6×10^6	$-.01 \times 10^6$
CP	3.5×10^5	$-.26 \times 10^5$	CU	1.5×10^6	$-.01 \times 10^6$
CG	4.8×10^5	$.49 \times 10^5$	CUD	1.3×10^6	$-.16 \times 10^6$
CC	3.3×10^5	$-.14 \times 10^5$	CB	1.9×10^6	$-.09 \times 10^6$
CP, CR	2.5×10^5	$-.04 \times 10^5$	CC, CU	1.8×10^6	$.11 \times 10^6$
CG, CR	3.0×10^5	$-.34 \times 10^5$	CC, CUD	1.6×10^6	$.06 \times 10^6$
CC, CR	3.7×10^5	$.09 \times 10^5$	CB, CC	1.5×10^6	$-.16 \times 10^6$
CG, CP	4.7×10^5	$-.26 \times 10^5$	CU, CUD	1.4×10^6	$.16 \times 10^6$
CC, CP	4.1×10^5	$-.49 \times 10^5$	CB, CU	1.4×10^6	$-.01 \times 10^6$
CC, CG	4.9×10^5	$-.64 \times 10^5$	CB, CUD	1.4×10^6	$.04 \times 10^6$
CG, CP, CR	4.6×10^5	$.66 \times 10^5$	CC, CU, CUD	1.5×10^6	$-.06 \times 10^6$
CC, CP, CR	3.5×10^5	$-.01 \times 10^5$	CB, CC, CU	1.3×10^6	$.06 \times 10^6$
CC, CG, CP	2.7×10^5	$-.79 \times 10^5$	CB, CU, CUD	1.5×10^6	$.16 \times 10^6$
CC, CB, CR	3.9×10^5	$.09 \times 10^5$	CB, CC, CUD	1.1×10^6	$.01 \times 10^6$
CC, CG, CP, CR	2.5×10^5	$-.21 \times 10^5$	CB, CC, CU, CUD	1.6×10^6	$.09 \times 10^6$
Control	2.9×10^5		Control	1.7×10^6	

* Denotes significance at 5% level of significance.

TABLE A6. THE EFFECTS OF VARIOUS CHLORO-ORGANIC CHEMICALS AT 0.1 ppm ON THE YIELD OF *PORPHYRIDIUM* SP.

Experiment 3			Experiment 4		
Treatment	Biomass (cells/ml)	Main effects & interactions	Treatment	Biomass (cells/ml)	Main effects & interactions
CR	2.5×10^5	$.65 \times 10^5$	CR	9.7×10^5	$.75 \times 10^5$ *
CP	6.8×10^5	$.75 \times 10^5$	CB	7.9×10^5	$-.38 \times 10^5$
CG	4.0×10^5	$.63 \times 10^5$	CUD	9.7×10^5	1.13×10^5 *
CU	4.8×10^5	$.15 \times 10^5$	CP	8.5×10^5	$-.70 \times 10^5$
CP, CR	2.5×10^5	$-.90 \times 10^5$	CB, CR	9.4×10^5	$-.58 \times 10^5$
CG, CR	5.2×10^5	$.98 \times 10^5$	CR, CUD	11.7×10^5	$.13 \times 10^5$
CR, CU	4.2×10^5	$.50 \times 10^5$	CP, CR	9.9×10^5	$-.70 \times 10^5$
CG, CP	5.5×10^5	$-.03 \times 10^5$	CB, CUD	9.9×10^5	$.25 \times 10^5$
CP, CU	4.7×10^5	$-.40 \times 10^5$	CB, CP	9.2×10^5	$-.03 \times 10^5$
CG, CU	3.5×10^5	$-.48 \times 10^5$	CP, CUD	9.0×10^5	$-.58 \times 10^5$
CG, CP, CR	5.0×10^5	$-.08 \times 10^5$	CB, CR, CUD	10.9×10^5	$.15 \times 10^5$
CP, CR, CU	4.1×10^5	$.35 \times 10^5$	CB, CP, CR	7.5×10^5	$-.38 \times 10^5$
CG, CP, CU	5.4×10^5	$.48 \times 10^5$	CB, CP, CUD	9.4×10^5	$.20 \times 10^5$
CG, CR, CU	4.9×10^5	$-.53 \times 10^5$	CP, CR, CUD	9.6×10^5	$.08 \times 10^5$
CG, CP, CR, CU	4.6×10^5	$-.48 \times 10^5$	CB, CR, CP, CUD	9.3×10^5	$.45 \times 10^5$
Control	3.5×10^5		Control	8.4×10^5	

* Denotes significance at 5% level of significance.

TABLE A7. THE EFFECTS OF VARIOUS CHLORO-ORGANIC CHEMICALS AT 0.1 ppm ON THE YIELD OF SCENEDESMUS OBLIQUUS

Experiment 1			Experiment 2		
Treatment	Biomass (cells/ml)	Main effects & interactions	Treatment	Biomass (cells/ml)	Main effects & interactions
CU	2.2×10^6	$.06 \times 10^6$	CG	1.9×10^6	$.04 \times 10^6$
CR	1.8×10^6	$.11 \times 10^6$	CUD	2.0×10^6	$-.04 \times 10^6$
CP	2.0×10^6	$.04 \times 10^6$	CC	1.7×10^6	$.01 \times 10^6$
CR, CU	1.8×10^6	$-.04 \times 10^6$	CB	2.0×10^6	$.04 \times 10^6$
CP, CU	1.9×10^6	$.01 \times 10^6$	CUD, CG	1.8×10^6	$-.09 \times 10^6$
CP, CR	1.6×10^6	$.04 \times 10^6$	CC, CG	2.0×10^6	$.01 \times 10^6$
CP, CR, CU	2.0×10^6	$.09 \times 10^6$	CB, CG	2.2×10^6	$.04 \times 10^6$
			CC, CUD	2.1×10^6	$.09 \times 10^6$
Control	2.0×10^6		CB, CUD	1.8×10^6	$-.14 \times 10^6$
			CB, CC	2.0×10^6	$-.04 \times 10^6$
			CC, CG, CUD	1.9×10^6	$.04 \times 10^6$
			CB, CG, CUD	1.8×10^6	$.11 \times 10^6$
			CB, CC, CUD	1.8×10^6	$.04 \times 10^6$
			CB, CC, CG	1.9×10^6	$-.04 \times 10^6$
			CB, CC, CUD, CG	2.0×10^6	$.09 \times 10^6$
			Control	1.8×10^6	

* Denotes significance at 5% level of significance.

TABLE A8. THE EFFECTS OF VARIOUS CHLORO-ORGANIC CHEMICALS AT 0.1 ppm ON THE YIELD OF SCENEDESMUS OBLIQUUS

Experiment 3			Experiment 4		
Treatment	Biomass (cells/ml)	Main effects & interactions	Treatment	Biomass (cells/ml)	Main effects & interactions
CU	1.9×10^6	$.01 \times 10^6$	CUD	8.2×10^5	$-.38 \times 10^5$
CP	2.0×10^6	$.04 \times 10^6$	CC	9.2×10^5	$-.13 \times 10^5$
CG	1.9×10^6	$-.09 \times 10^6$	CR	8.2×10^5	$-.13 \times 10^5$
CB	2.1×10^6	$.01 \times 10^6$	CP	8.8×10^5	$.43 \times 10^5$
CP, CU	2.2×10^6	$-.04 \times 10^6$	CC, CUD	8.8×10^5	$-.13 \times 10^5$
CG, CU	2.0×10^6	$.09 \times 10^6$	CR, CUD	8.8×10^5	$-.03 \times 10^5$
CB, CU	1.9×10^6	$-.06 \times 10^6$	CP, CUD	8.8×10^5	$.28 \times 10^5$
CG, CP	1.9×10^6	$-.09 \times 10^6$	CC, CR	8.2×10^5	$.48 \times 10^5$
CB, CP	2.2×10^6	$-.04 \times 10^6$	CC, CP	9.2×10^5	$.23 \times 10^5$
CB, CG	1.8×10^6	$-.01 \times 10^6$	CP, CR	10.0×10^5	$.28 \times 10^5$
CP, CG, CU	1.9×10^6	$.06 \times 10^6$	CC, CR, CUD	8.2×10^5	$.08 \times 10^5$
CB, CP, CU	1.9×10^6	$-.06 \times 10^6$	CC, CP, CUD	8.8×10^5	$-.03 \times 10^5$
CB, CG, CP	1.9×10^6	$.04 \times 10^6$	CC, CP, CR	9.2×10^5	$.08 \times 10^5$
CB, CG, CU	2.1×10^6	$.11 \times 10^6$	CP, CR, CUD	9.0×10^5	$.44 \times 10^5$
CG, CG, CP, CU	1.9×10^6	$.01 \times 10^6$	CC, CP, CR, CUD	8.0×10^5	$.13 \times 10^5$
Control	1.9×10^6		Control	8.8×10^5	

* Denotes significance at 5% level of significance.

TABLE A9. THE EFFECTS OF VARIOUS CHLORO-ORGANIC CHEMICALS AT 0.1 ppm ON THE YIELD OF *SELENASTRUM CAPRICORNUTUM*

Experiment 1			Experiment 2		
Treatment	Biomass (cells/ml)	Main effects & interactions	Treatment	Biomass (cells/ml)	Main effects & interactions
CU	6.3×10^6	-0.14×10^6	CG	4.0×10^6	$.09 \times 10^6$
CR	6.1×10^6	$.11 \times 10^6$	CUD	3.6×10^6	$-.21 \times 10^6$
CP	5.9×10^6	$.24 \times 10^6$	CC	4.1×10^6	$.01 \times 10^6$
CR, CU	6.1×10^6	$.01 \times 10^6$	CB	4.1×10^6	$-.11 \times 10^6$
CP, CU	4.9×10^6	$.24 \times 10^6$	CG, CUD	3.7×10^6	$.09 \times 10^6$
CP, CR	6.1×10^6	$.11 \times 10^6$	CC, CG	5.2×10^6	$.06 \times 10^6$
CP, CR, CU	5.6×10^6	$.11 \times 10^6$	CB, CG	3.7×10^6	$-.16 \times 10^6$
			CC, CUD	3.9×10^6	$-.24 \times 10^6$
Control	5.9×10^6		CB, CUD	4.1×10^6	$.39 \times 10^6$
			CB, CC	4.1×10^6	$-.39 \times 10^6$
			CC, CG, CUD	4.0×10^6	$-.29 \times 10^6$
			CB, CG, CUD	4.8×10^6	$.24 \times 10^6$
			CB, CC, CUD	3.8×10^6	$-.14 \times 10^6$
			CB, CC, CG	3.7×10^6	$-.29 \times 10^6$
			CB, CC, CG, CUD	3.6×10^6	$.06 \times 10^6$
			Control	4.3×10^6	

* Denotes significance at 5% level of significance.

TABLE A10. THE EFFECTS OF VARIOUS CHLORO-ORGANIC CHEMICALS AT 0.1 ppm ON THE YIELD OF SELENASTRUM CAPRICORNUTUM

Experiment 3			Experiment 4		
Treatment	Biomass (cells/ml)	Main effects & interactions	Treatment	Biomass (cells/ml)	Main effects & interactions
CU	4.3×10^6	$.13 \times 10^6$	CUD	2.2×10^6	$-.05 \times 10^6$
CP	4.3×10^6	$-.15 \times 10^6$	CC	2.4×10^6	$.03 \times 10^6$
CG	4.5×10^6	$.03 \times 10^6$	CR	2.5×10^6	$.00 \times 10^6$
CB	4.3×10^6	$-.08 \times 10^6$	CP	2.6×10^6	$.05 \times 10^6$
CP, CU	4.3×10^6	$.08 \times 10^6$	CC, CUD	2.5×10^6	$.05 \times 10^6$
CG, CU	4.9×10^6	$.05 \times 10^6$	CR, CUD	2.5×10^6	$.03 \times 10^6$
CB, CU	4.5×10^6	$-.05 \times 10^6$	CP, CUD	2.4×10^6	$-.03 \times 10^6$
CG, CP	4.0×10^6	$-.08 \times 10^6$	CR, CC	2.4×10^6	$-.05 \times 10^6$
CB, CP	4.2×10^6	$.13 \times 10^6$	CC, CP	2.5×10^6	$.00 \times 10^6$
CB, CG	4.4×10^6	$-.10 \times 10^6$	CP, CR	2.4×10^6	$-.08 \times 10^6$
CG, CP, CU	4.5×10^6	$.00 \times 10^6$	CC, CR, CUD	2.4×10^6	$-.08 \times 10^6$
CB, CP, CU	4.5×10^6	$.00 \times 10^6$	CC, CP, CUD	2.5×10^6	$-.03 \times 10^6$
CB, CG, CP	4.3×10^6	$.10 \times 10^6$	CC, CP, CR	2.5×10^6	$.08 \times 10^6$
CB, CG, CU	4.2×10^6	$-.23 \times 10^6$	CP, CR, CUD	2.4×10^6	$.00 \times 10^6$
CB, CG, CP, CU	4.3×10^6	$.03 \times 10^6$	CC, CP, CR, CUD	2.4×10^6	$.00 \times 10^6$
Control	4.5×10^6		Control	2.4×10^6	

* Denotes significance at 5% level of significance.

TABLE A11. THE EFFECTS OF VARIOUS CHLORO-ORGANIC CHEMICALS AT 0.1 ppm ON THE YIELD OF SKELETONEMA COSTATUM

Experiment 1			Experiment 2		
Treatment	Biomass (cells/ml)	Main effects & interactions	Treatment	Biomass (cells/ml)	Main effects & interactions
CR	5.0×10^5	-1.00×10^5*	CR	3.3×10^5	$-.21 \times 10^5$ *
CP	4.5×10^5	$-.30 \times 10^5$	CB	3.3×10^5	$-.26 \times 10^5$ *
CG	4.6×10^5	$.05 \times 10^5$	CUD	3.1×10^5	$-.50 \times 10^5$ *
CC	2.7×10^5	-1.45×10^5 *	CP	2.8×10^5	$-.69 \times 10^5$ *
CP, CR	3.2×10^5	$-.50 \times 10^5$ *	CB, CR	2.6×10^5	$.21 \times 10^5$
CG, CR	4.0×10^5	$-.30 \times 10^5$	CR, CUD	2.8×10^5	$.31 \times 10^5$ *
CC, CR	1.7×10^5	$-.10 \times 10^5$	CP, CR	2.6×10^5	$.39 \times 10^5$ *
CG, CP	4.5×10^5	$.00 \times 10^5$	CB, CUD	2.8×10^5	$.26 \times 10^5$ *
CC, CP	3.0×10^5	$.60 \times 10^5$*	CB, CP	2.6×10^5	$.34 \times 10^5$ *
CC, CG	3.2×10^5	$.50 \times 10^5$*	CP, CUD	2.1×10^5	$.09 \times 10^5$
CG, CP, CR	2.3×10^5	$-.05 \times 10^5$	CB, CR, CUD	2.6×10^5	$.04 \times 10^5$
CC, CP, CR	2.0×10^5	$.35 \times 10^5$	CB, CP, CR	2.6×10^5	$.06 \times 10^5$
CC, CG, CP	3.8×10^5	$.00 \times 10^5$	CB, CP, CUD	1.9×10^5	$-.09 \times 10^5$
CC, CG, CR	2.3×10^5	$.20 \times 10^5$	CP, CR, CUD	2.1×10^5	$-.04 \times 10^5$
CC, CG, CP, CR	2.3×10^5	$-.10 \times 10^5$	CB, CP, CR, CUD	2.8×10^5	$.14 \times 10^5$
Control	4.5×10^5		Control	4.5×10^5	

* Denotes significance at 5% level of significance.

TABLE A12. THE EFFECTS OF VARIOUS CHLORO-ORGANIC CHEMICALS AT 0.1 ppm ON THE YIELD OF SKELETONEMA COSTATUM

Experiment 3			Experiment __		
Treatment	Biomass (cells/ml)	Main effects & interactions	Treatment	Biomass (cells/ml)	Main effects & interactions
CR	4.0×10^5	$.24 \times 10^5$			
CP	3.8×10^5	$-.41 \times 10^5$			
CG	4.3×10^5	$.69 \times 10^5$			
CU	4.5×10^5	$.19 \times 10^5$			
CP, CR	3.8×10^5	$-.01 \times 10^5$			
CG, CR	4.5×10^5	$-.61 \times 10^5$			
CR, CU	4.5×10^5	$.24 \times 10^5$			
CG, CP	5.0×10^5	$.04 \times 10^5$			
CP, CU	2.1×10^5	$-.56 \times 10^5$			
CG, CU	5.0×10^5	$-.06 \times 10^5$			
CG, CP, CR	4.0×10^5	$-.46 \times 10^5$			
CP, CR, CU	4.7×10^5	$.49 \times 10^5$			
CG, CP, CU	4.5×10^5	$.09 \times 10^5$			
CG, CR, CU	5.0×10^5	$-.21 \times 10^5$			
CG, CP, CR, CU	3.8×10^5	$-.36 \times 10^5$			
Control	3.2×10^5				

* Denotes significance at 5% level of significance.

TABLE A13. THE EFFECTS OF VARIOUS CHLORO-ORGANIC CHEMICALS AT 0.1 ppm ON THE YIELD OF THALASSIOSIRA PSEUDONANA

Experiment 1			Experiment 2		
Treatment	Biomass (cells/ml)	Main effects & interactions	Treatment	Biomass (cells/ml)	Main effects & interactions
CR	2.6×10^6	$-.13 \times 10^6$	CC	2.5×10^6	$-.03 \times 10^6$
CP	3.1×10^6	$.18 \times 10^6$ *	CU	2.3×10^6	$-.13 \times 10^6$
CG	3.1×10^6	$-.18 \times 10^6$ *	CUD	2.6×10^6	$.08 \times 10^6$
CC	3.0×10^6	$-.13 \times 10^6$	CB	2.5×10^6	$.08 \times 10^6$
CP, CR	3.1×10^6	$.23 \times 10^6$ *	CC, CU	2.2×10^6	$.05 \times 10^6$
CG, CR	2.7×10^6	$.03 \times 10^6$	CC, CUD	2.3×10^6	$-.10 \times 10^6$
CC, CR	2.6×10^6	$.03 \times 10^6$	CB, CC	2.8×10^6	$.05 \times 10^6$
CG, CP	2.8×10^6	$-.18 \times 10^6$ *	CU, CUD	2.6×10^6	$.10 \times 10^6$
CC, CP	3.1×10^6	$.03 \times 10^6$	CB, CU	2.4×10^6	$-.10 \times 10^6$
CC, CG	2.8×10^6	$-.18 \times 10^6$ *	CB, CUD	2.8×10^6	$-.10 \times 10^6$
CG, CP, CR	3.1×10^6	$.03 \times 10^6$	CC, CU, CUD	2.7×10^6	$.13 \times 10^6$
CC, CP, CR	3.3×10^6	$.03 \times 10^6$	CB, CC, CU	2.5×10^6	$-.03 \times 10^6$
CC, CG, CD	2.6×10^6	$-.03 \times 10^6$	CB, CU, CUD	2.4×10^6	$-.13 \times 10^6$
CC, CG, CR	2.5×10^6	$-.03 \times 10^6$	CB, CC, CUD	2.5×10^6	$-.08 \times 10^6$
CC, CG, CP, CR	2.6×10^6	$-.08 \times 10^6$	CB, CC, CU, CUD	2.4×10^6	$.00 \times 10^6$
Control	2.9×10^6		Control	2.5×10^6	

* Denotes significance at 5% level of significance.

TABLE A14. THE EFFECTS OF VARIOUS CHLORO-ORGANIC CHEMICALS AT 0.1 ppm ON THE YIELD OF THALASSIOSIRA PSEUDONANA

Experiment 3			Experiment 4		
Treatment	Biomass (cells/ml)	Main effects & interactions	Treatment	Biomass (cells/ml)	Main effects & interactions
CR	3.1×10^6	$-.14 \times 10^6$	CR	3.4×10^6	$-.20 \times 10^6$
CP	3.4×10^6	$-.06 \times 10^6$	CB	3.4×10^6	$-.18 \times 10^6$
CG	3.1×10^6	$-.26 \times 10^6$ *	CUD	3.4×10^6	$-.23 \times 10^6$
CU	3.0×10^6	$.21 \times 10^6$	CP	3.6×10^6	$.28 \times 10^6$
CP, CR	3.0×10^6	$-.04 \times 10^6$	CB, CR	3.4×10^6	$.18 \times 10^6$
CG, CR	2.8×10^6	$.09 \times 10^6$	CR, CUD	3.6×10^6	$.23 \times 10^6$
CU, CR	3.1×10^6	$.01 \times 10^6$	CP, CR	3.5×10^6	$.18 \times 10^6$
CG, CP	2.8×10^6	$-.01 \times 10^6$	CG, CUD	3.7×10^6	$.25 \times 10^6$
CP, CU	2.8×10^6	$-.06 \times 10^6$	CB, CP	3.6×10^6	$.15 \times 10^6$
CG, CU	2.8×10^6	$-.01 \times 10^6$	CP, CUD	3.3×10^6	$.00 \times 10^6$
CG, CP, CR	2.9×10^6	$.06 \times 10^6$	CB, CR, CUD	3.6×10^6	$-.25 \times 10^6$
CP, CR, CU	2.9×10^6	$-.04 \times 10^6$	CB, CP, CR	3.6×10^6	$-.15 \times 10^6$
CG, CP, CU	2.9×10^6	$.09 \times 10^6$	CB, CP, CUD	3.3×10^6	$-.23 \times 10^6$
CG, CR, CU	2.6×10^6	$-.14 \times 10^6$	CP, CR, CUD	3.3×10^6	$-.20 \times 10^6$
CG, CP, CR, CU	2.4×10^6	$-.14 \times 10^6$	CB, CP, CR, CUD	3.3×10^6	$.23 \times 10^6$
Control	3.1×10^6		Control	5.0×10^6	

* Denotes significance at 5% level of significance.

TABLE A15. REPRESENTATIVE DATA COMPARING CELL NUMBERS OBTAINED BY DIRECT COUNTING AND FROM A CALIBRATION CURVE FOR PORPHYRIDIUM SP.

Cells/ml	
Direct cell count[1/]	Calibration curve[2/]
1.0×10^5	1.2×10^5
3.2×10^5	3.2×10^5
3.6×10^5	3.6×10^5
8.0×10^5	9.0×10^5
1.1×10^6	9.6×10^5
1.1×10^6	1.1×10^6
1.1×10^6	1.2×10^6
1.2×10^6	1.4×10^6
2.0×10^6	2.1×10^6
2.5×10^6	2.6×10^6

1/ Direct cell count made with an A-0 Bright-Line hemacytometer.

2/ Calibration curve related cell number to optical density measurements.